Report

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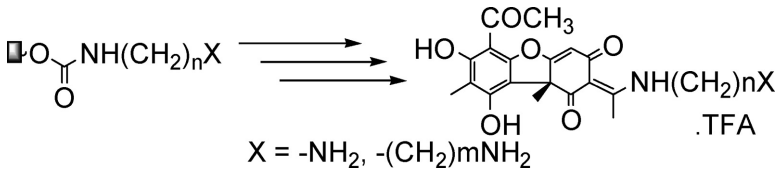

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Solid-Phase Synthesis of Polyfunctionalized Natural Products: Application to Usnic Acid, a Bioactive Lichen Compound

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In a recent review, Koehn and Carter detailed the important role and the renewed interest in natural products in drug discovery, particularly as building blocks for molecular libraries.[1] In this way, a parallel approach could be developed to an elaborate target-oriented or focused library involving structural modification onto an existing bioactive natural product scaffold to improve inherent biological activity. Similarly, technology as combinatorial chemistry is known as an essential tool for drug discovery.[2] We have reported here an efficient methodology for modulation of a natural bioactive compound in a parallel manner which could be applied to other natural polyfunctionalized products. The existence of various functionalities required the selection of the appropriate strategy to afford diversity with easy purification.

Thus, this paper deals with the parallel synthesis of a small set based on a natural lichen dibenzofuran derivative, usnic acid (Figure 1). This polyfunctionalized compound exhibited several activities.[3,4] One of the most interesting activities concerned an antibacterial effect against some Gram-positive (*Staphylococcus aureus*, *Enteroccus faecalis*, *Streptococcus* species) and against some anaerobic bacteria and mycobacteria. Usnic acid is thus an interesting target for structural modification. In this way, the antibacterial activity of mono- and diacetates[5,6] or amino derivatives[7−12] has been already reported. Although the acetylation of the hydroxy groups decreased the activity against *S. aureus*, potent activities were noted against *Mycobacterium tuberculosis* for diacetate, thiosemicarbazone, and sulfaguanidine derivatives.[5−10]

Polyamines present interesting features, such as (i) an easy supply of diversity, (ii) an affinity for nucleic acids, and (iii) a potential increase of activity in various fields when they are conjugated to drugs.[13] Thus, introduction of a polycationic side chain provided teicoplanin derivatives with an increase in basic character, hydrosolubility, and activity against Gram-positive and Gram-negative bacteria.[14] In exploring the antibacterial potential of new usnic acid derivatives, it seemed interesting to develop condensation of polyamines with usnic acid to improve antibacterial activity and to overcome the poor hydrosolubility of usnic acid.[15] As a class of natural broad-spectrum antibiotics, glycocinnamoylspermidines (derived from a triamine, Figure 1)[16] has been described. We prepared di- and triamine derivatives of usnic acid and evaluated them against some Gram-positive bacteria.

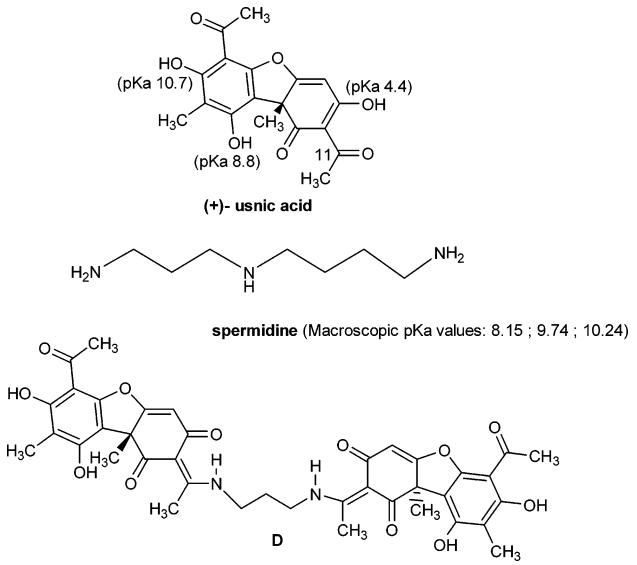

Figure 1. Structure of (+)-usnic acid; a triamine/spermidine; and a dimeric product of usnic acid, **D**.

First attempts for condensation of diaminopropane with usnic acid had resulted in the following observations: (i) the use of equimolar amounts of diamine afforded adduct compounds that are very difficult to separate to the expected product, and (ii) an excess led to an inactive dimeric product of usnic acid D (Figure 1). Thus, a supplementary preparation step of mono-Boc-protected polyamine was necessary but impeded the rapid obtention of a collection of di- or triamine derivatives.

To overcome these problems and to generate rapidly our series of usnic acid derivatives, a parallel supported synthesis has thus been developed using Multipin SynPhase supports, which offer attractive features already known for SPOS.[17] Some advantages are particularly convenient for the preparation of our derivatives, namely, (i) preparation of various polyamines by a split-and-mix method;[18,19] (ii) primary amine protection, avoiding preliminary protective steps necessary in solution-phase synthesis; and (iii) simple purification by adapted washing of supports, whereas purification of highly polar polyamines during conventional synthesis is extremely tedious. The strategy used has consisted of condensing usnic acid to various di- or triamines preliminarily attached to the polymer. The triamine derivatives were prepared by a sequential solid-phase synthesis based on a previously reported methanesulfonate displacement strategy.[18]

The synthesis of usnic acid derivatives **33**−**56** (triamines) and **3**−**8** (diamines) is shown in Scheme 1. SynPhase

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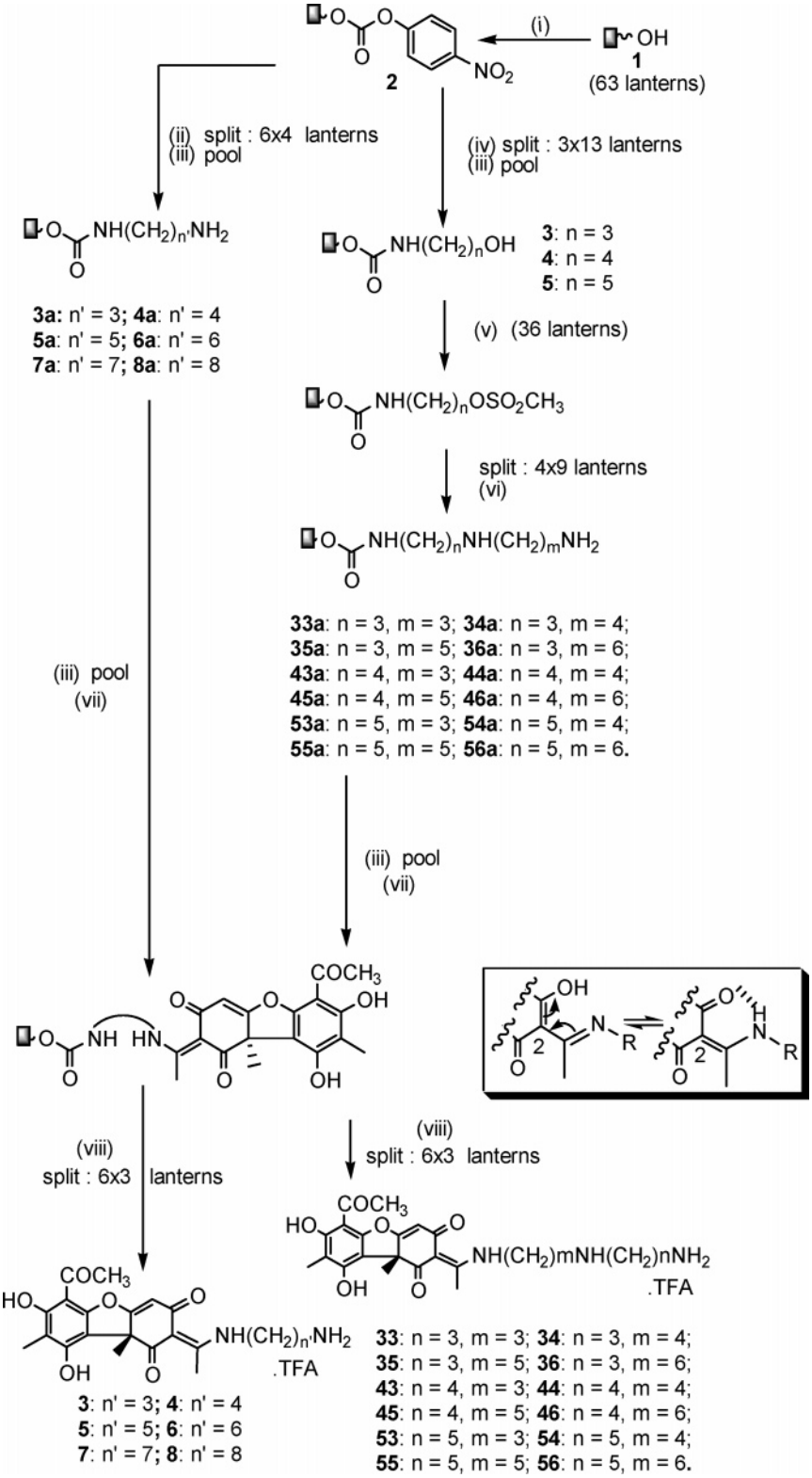
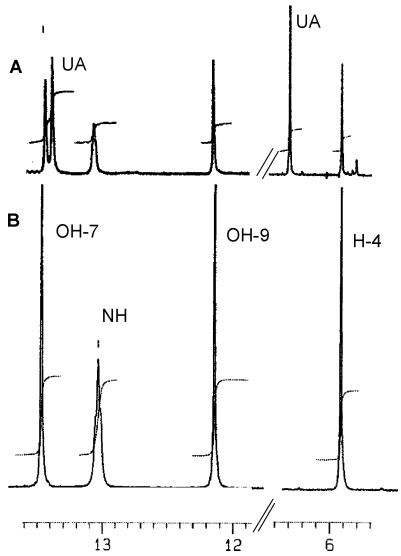
Scheme 1. Synthetic Route toward Usnic Acid Derivatives **33**−**56** and **3**−**8**[a]



[a] Reagents and conditions: (i) 4-Nitrophenylchloroformate 0.2 mol L^{-1}, 4-methylmorpholine 0.2 mol L^{-1}, CH_2Cl_2, rt, 45 min; (ii) $H_2N-(CH_2)_{n'}-NH_2$ ($n' = 3-8$), 1 mol L^{-1}, CH_2Cl_2, rt, 2 h; (iv) $H_2N-(CH_2)_n-OH$ ($n = 3-5$), 1 mol L^{-1}, CH_2Cl_2, rt, 2 h; (v) methanesulfonyl chloride 1.1 mol L^{-1}, pyridine, rt, 30 min; (vi) $H_2N-(CH_2)_m-NH_2$ ($m = 3-6$), 1 mol L^{-1}, DMSO, 50 °C, 6 h; (vii) usnic acid 0.05 mol L^{-1}, THF/EtOH 50:50, 50 °C, 5 h. (viii) TFA/CH_2Cl_2, 20:80, rt, 1 h.

supports **1** (63) were activated under anhydrous conditions with 4-nitrophenylchloroformate in the presence of *N*-methylmorpholine (0.2 mol L^{-1}, 45 min, CH_2Cl_2), then reacted with appropriate commercial amino alcohols ($n = 3-5$) or diamines ($n = 3-8$) (0.4 mol L^{-1}, 2 h, CH_2Cl_2), affording supports **3**−**5** and **3a**−**8a**, respectively.[20] For each step, one support was cleaved using TFA/CH_2Cl_2 20:80 in the presence of 4-nitrophenol (35 μmol), which was used as internal standard to lead to the evaluation of the yield and purity by ^{1}H NMR of cleaved products **3.s**−**5.s** and **3a.s**−

Scheme 2. Selected Part of ^{1}H NMR Spectra of Cleaved Products Resulting from **33a** and Usnic Acid Condensation[a]



[a] (A) Crude cleaved products, UA, signals of usnic acid; (B) cleaved product after acidic washing supports.

8a.s.[18] Although the reaction was complete with amino alcohols, diamines were grafted with a 60−74% range of yield, indicating the existence of an initial double-binding (cross-linkage) to the polymer for the latter. The supports **3**−**5** were pooled for the mesylation (methanesulfonyl chloride, 1.1 mol L^{-1}, 30 min, pyridine) then divided into four pools for the nucleophilic displacement by various diamines ($n = 3-6$) (1 mol L^{-1}, 6 h, DMSO). The resulting supported triamines **33a**−**56a** were prepared with a 34−49% range of yield and a purity >90%. In agreement with our previous observations, the loss of yield during the displacement step, which seemed to increase with the decreasing of chain length of mesylated amino alcohols, came from the intramolecular cyclization (attack of mesylated group on carbamate) affording cleaved cyclic urethanes.[19]

The supports **33a**−**56a** and **3a**−**8a** were separately mixed in two batches for the condensation with usnic acid. The best conditions for this step on SPOS were preliminarily determined by analysis of cleaved products by HPLC using 4-nitrobenzene as internal standard and involved the use of usnic acid (0.05 mol L^{-1}, 5 h) in THF/EtOH 50:50. No protection of the secondary amine of the supported triamines was apparently necessary because of the regioselectivity of the reaction of amine with usnic acid. In fact, the reaction occcurs at the C-11 acetyl group, affording an initial imine product that undergoes rapid tautomerization to the more stable enamine system (Scheme 1).[21] This final condensation product was stabilized by the existence of a hydrogen bond between the NH and the carbonyl group at position 3 and could not be formed from an imine resulting from the reaction between a secondary amine and the acetyl group. In the same way, the condensation product possibility formed at C-13 was then hydrolyzed during the washing step; however, a byproduct was observed after cleavage of the supported products afforded by the condensation of **33a**−**56a** and usnic acid (Scheme 1). This product was usnic acid and resulted, regardless the pK_a values[22,23] (Figure 1), from the salt formation between the NH of supported triamines **33a**−**56a** and one enol group of usnic acid, in excess. In comparison with solution steps, however, no additional protection step was necessary. After various attempts, an additional washing step was thus easily performed on supports **33a**−**56a** to eliminate this product before final cleavage using either Et_3N (0.05 mol L^{-1}) then HCOOH 5% in THF or, more simply, HCOOH 5% in THF over 45 min.[24]

Finally, supports **33a**−**56a** and **3a**−**8a** were cleaved in acidic conditions to give after purification steps the expected usnic acid derivatives **33**−**56** and **3**−**8** in reasonable yield (30−40% and 17−50%, respectively) and purity (>87%). In fact, ^{1}H NMR spectra of cleaved compounds showed the presence of free di- and triamines resulting from the cross-linkage for diamines and for both series of an incomplete condensation with usnic acid, which was too bulky to hit all free NH_2 groups. An additional purification step was easily accomplished by washing the dichloromethane solution of diamine compounds with water (150 μL). The highly polar triamine derivatives needed a purification step using column chromatography on a Sephadex LH20 column with methanol as eluent. The ^{1}H, ^{13}C, and 2D NMR and HRMS spectra of all final compounds supported the valuation of good purity.

The antibacterial activity was estimated by agarose diffusion assay[25] not only against *S. aureus* and *E. faecalis*, which were sensitive to usnic acid, but also against *Listeria monocytogenes*, which causes a rare but serious disease. The significant activities are reported in Table 1. By comparison with usnic acid, the addition of amine chain improved the activity against *S. aureus*, which supported our idea to enhance activity with amines, due to an increase in hydrosolubility. The best activities were observed for the triamine derivatives **33**, **43**, and **53** and for one diamine, **3**, indicating the importance of the aminopropyl chain. The least activity of analogous derivatives **34** and **35** confirmed these observations. An enhanced activity was noted against *L. monocytogenes* for the most of our derivatives ($d_{inhi} = 12-14$ mm). However, a total loss of activity against *E. faecalis* was shown for our derivatives in comparison with the one of usnic acid ($d_{inhi} = 20$ mm). Our derivatives were, thus, more selective than usnic acid, with a good activity on *L. monocytogenes*. Moreover, no increase in activity was shown against Gram-negative bacteria, which indicates an inability of usnic acid and its derivatives to pass through the lipopolysaccharide membrane of these bacteria.

Table 1. Antibacterial Activity of Usnic Acid, Usnic Acid Derivatives **33**−**56**, **3**−**8** and **D**[a]

	compd (50 μg)																			
	usnic acid	**33**	**34**	**35**	**36**	**43**	**44**	**45**	**46**	**53**	**54**	**55**	**56**	**3**	**4**	**5**	**6**	**7**	**8**	**D**
S. aureus	10	**13**	11	11	10	**14**	11	11	11	12	12	12	11	12	9	9	9	7	7	6
L. monocytogenes	10	**14**	12	12	11	**14**	12	12	12	**13**	12	**13**	12	**13**	12	12	11	8	9	6

[a] d_{inhi}, mm: diameter of inhibition zone of bacterial growth.

In conclusion, we have developed an efficient and rapid parallel synthesis of polyamine usnic acid derivatives involving polyfunctionalized and polar substrates. This versatile methodology avoided heavy purification steps and led to the preparation of a sufficient amount (10 mg) of 18 pure hydrosoluble compounds for rapid antibacterial screening. This preliminary assay has shown the aminopropyl group to be involved in this activity.

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Supporting Information Available. Details of experimental procedures and spectroscopic data and antibacterial procedures for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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