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Organic reactions in frozen water: Michael addition of amines and thiols to the dehydroalanine side chain of nocathiacins ☆, ☆☆

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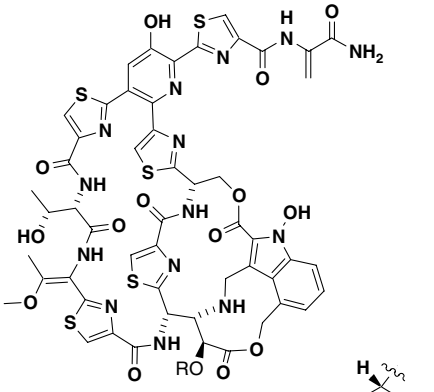
Abstract—Nocathiacins are densely functionalized cyclic thiazolyl peptide natural products with potent in vitro and in vivo antibacterial activity against a variety of gram-positive bacteria, including a number of multiple drug-resistant strains. Attempts to prepare Michael adducts using known conditions resulted in the formation of complex mixture of products. In order to overcome this problem, we developed unique conditions in which Michael addition of amine and thiol nucleophiles to the dehydroalanine moiety of nocathiacins was successfully achieved in frozen water. Under these conditions, the Michael addition was highly chemoselective, very efficient and provided good isolated yields of the desired products.

Nocathiacins are a class of cyclic thiazolyl peptide antibiotics isolated from the fermentation broth of *Nocardia* sp.[2] and *Amycolatopsis* sp.[3] These compounds display potent antibacterial activity against a variety of gram-positive bacteria, including a number of multiple drug-resistant strains such as methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Enterococcus faecium*, and penicillin-resistant *Streptococcus pneumoniae*. Most importantly, these nocathiacins possess desirable bactericidal activity. Unlike some other thiazolyl peptide antibiotics such as nosiheptide, nocathiacins also show in vivo efficacy in a mouse systemic *S. aureus* infection model. The nocathiacins are distinguished from other thiazolyl peptides by the presence of an indole ring within the cyclic scaffold.[4] The indole is attached to the main cyclic framework by ester and ether linkages, both of which are chemically labile. The tricyclic framework of the nocathiacins is required for the biological activity: opening of one or both of the macrolactone rings results in inactive compounds. In this paper, we describe new, mild, and useful conditions to the Michael addition of amines and thiols to the dehydroalanine moiety of nocathiacins in frozen water.



As a part of our effort to develop new antibacterial agents active against resistance bacteria and suitable for intravenous (iv) administration, we undertook an

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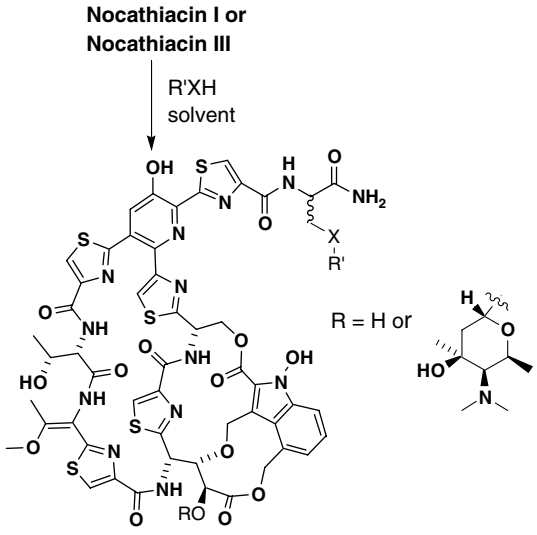
investigation to modify the nocathiacins to improve their aqueous solubility while maintaining their intrinsic biological activity. One of our approaches was to introduce polar, water-solubilizing groups into the molecule through chemical transformation.[5]

The delicate nature of the nocathiacins coupled with their dense functionality necessitated development of extremely mild and highly selective conditions to effect the desired transformations. During the course of our work, we investigated Michael addition[6] of amines and thiols to the dehydroalanine side chain as a means of introducing a water-solubilizing functionality (Scheme 1). Surprisingly, there are only a handful of reports on the Michael addition of nucleophiles to dehydroalanine derivatives,[7] most likely because of the moderate reactivity of the conjugated system.[7d]

We began our studies using methanol as a solvent because the nocathiacins are sparingly soluble in commonly used organic solvents such as THF and CH_2Cl_2, but are soluble in methanol. In addition, it is well established that the Michael addition works well in protic solvents.[8] To our disappointment, treatment of nocathiacin I with methylamine in methanol gave multiple products after 1 h at room temperature (Table 1, entry 1 and 2). It appeared that the initially formed Michael adduct decomposed to a complex mixture of products under these conditions.



Scheme 1.

We then considered the use of water as a solvent for a variety of reasons. First, although nocathiacins have extremely low solubility at neutral pH, we thought that the hydroxypyridine moiety would assist in solubilizing these compounds at higher pH.[9] Second, water might accelerate the formation of the desired product with fewer side products. Even though use of water as a reaction solvent has received considerable attention in synthetic organic chemistry,[10] Michael additions in water are relatively scarce,[11,12] and it may be possible to realize reactivity and/or selectivity in water that cannot be attained in organic solvents.[13]

In the event, treatment of nocathiacin I with 10 equiv of methylamine in water proceeded smoothly to provide the Michael adduct in good yield in 1 h (Table 1, entry 3). Mass spectral data coupled with the absence of the distinct vinyl proton singlet signals at 6.37 and 5.76 ppm of dehydroalanine unit provides unequivocal evidence for the Michael adduct. Under these conditions, dimethylamine also underwent Michael addition to nocathiacin I to furnish the adduct in good yield in 1 h (Table 1, entry 4). However, the addition of morpholine to nocathiacin I was much slower and was not complete even after 24 h (Table 1, entry 5). Furthermore, in addition to the desired Michael adduct several unidentified side products were formed. We rationalized that lowering the reaction temperature might suppress the undesired side reactions and improve the overall outcome of the desired process. Thus, the Michael addition of morpholine to nocathiacin I proceeded in higher yield and with fewer side products at 5 °C (Table 1, entry 6). Most importantly, and to our surprise, the best results were obtained when a homogeneous aqueous solution of nocathiacin I and morpholine was frozen solid at −20 °C. The Michael addition was complete in 5.5 h and gave the product in 90% yield (Table 1, entry 7).[14] By comparison, a solution of nocathiacin I and morpholine in 1:4 MeOH/H_2O remained liquid when cooled to −20 °C. However, even after 7 days, 27% of nocathiacin I remained unreacted, although no unwanted side products were formed under these conditions. The data from

Table 1. Optimization of Michael addition with nocathiacin I[a]

Entry	Amine/equiv (R′XH)	Solvent	T (°C)	Time (h)	Yield [b](%)
1	$MeNH_2$/25	MeOH	22	3	—[c]
2	$MeNH_2$/10	MeOH	22	7	—[c]
3	$MeNH_2$/10	H_2O	22	1	70 (5)[d]
4	Me_2NH/10	H_2O	22	1	76
5	Morpholine/10	H_2O	22	24	50 (14)[d]
6	Morpholine/10	H_2O	5	24	70 (14)[d]
7	Morpholine/10	H_2O	−20	5.5	90 (3)[d]
8	Morpholine/10	1:4 MeOH/H_2O	−20	120	70 (27)[d]

[a] Reactions were conducted on 0.10 mmol scale in 5 mL of solvent.
[b] Determined by HPLC analysis of crude reaction mixture.
[c] Multiple products were formed.
[d] Numbers in parentheses are the percentage of unreacted nocathiacin I.

Table 2. Michael addition of amines and thiols to nocathiacins[a]

Entry	Nocathiacin	Nucleophile (R′XH)	Yield[b] (%)
1	I	Methylamine	83 (55)
2	I	2-(2′-Aminoethyl)pyridine	72 (46)
3	I	1-(3′-Aminopropyl)imidazole	83 (63)
4	III	1-(3′-Aminopropyl)imidazole	56 (27)
5	I	Dimethylamine	89 (53)
6	I	*N*-Methylaminoethanol	88 (57)
7	I	*N*,*N*-Diethyl-*N*′-methylethylenediamine	83 (45)
8	I	*N*,*N*,*N*′-Trimethylethylenediamine	86 (44)
9	I	Morpholine	90 (43)
10	I	*N*-Methylpiperazine	91 (41)
11	I	Pyrrolidine	84 (57)
12	I	Glycine[d]	Trace[c]
13	I	Glycine methyl ester[d]	Trace[c]
14	I	β-Alanine[d]	Trace[c]
15	I	2-(*N*,*N*-Dimethylamino)ethanethiol[d]	97 (83)
16	I	Mercaptoacetic acid[d]	89 (64)
17	III	Mercaptoacetic acid[d]	70 (28)
18	I	5-Mercaptoethyl-tetrazole[d,e]	56 (15)
19	I	2-Mercaptoethanesulfonic acid[d,e]	85 (70)

[a] Unless otherwise mentioned the reactions were conducted using 10 equiv of amine or 10 equiv of thiol and Et_3N.
[b] Determined by HPLC analysis of crude reaction mixture; the numbers in parentheses are the isolated yields.
[c] The presence of products was only seen by MS.
[d] The Michael additions were conducted in the presence of Et_3N.
[e] Thiol (5 equiv) and Et_3N was used.

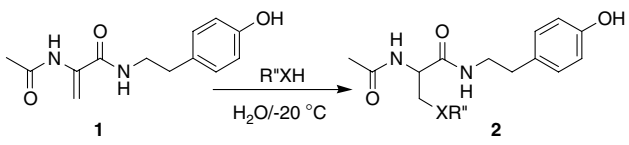
entries 5–8 in Table 1 clearly suggests that at −20 °C, processes leading to undesired side products were inhibited.

We next employed a variety of amines in the Michael addition at −20 °C in order to determine the generality of the reaction conditions (Table 2, entries 1–14). Both primary and secondary amines reacted smoothly with nocathiacins to give the corresponding Michael adducts in moderate isolated yields (Table 2, entries 1–8). Similarly, cyclic amines such as morpholine, *N*-methylpiperazine and pyrrolidine effectively underwent Michael addition to furnish adducts in respectable isolated yields (Table 2, entries 9–11). However, the conjugate addition of amino acids such as glycine, glycine methyl ester, and β-alanine was extremely slow: after 24 h only trace amount of corresponding Michael adducts were detected by mass spectroscopy (entries 12–14).

For the Michael addition of thiols to nocathiacins, inclusion of triethylamine was essential to facilitate the reaction. The added base plays a dual role of improving the solubility of nocathiacins and acting as a base catalyst for Michael addition of thiols. We found that thiols containing both basic and acidic functional groups reacted very efficiently with nocathiacins to give the corresponding Michael adducts in moderate to good isolated yields (Table 2, entries 15–19).

Although both nocathiacin I and III have undergone Michael addition in high conversion yields, in several cases the desired products were isolated only in moderate yields. We believe the profound affinity of these compounds for the chromatographic stationary phase as well as degradation during purification may be contributing to the low recovery. Michael adducts were generated as 1:1 mixture of diastereomers despite the fact that the nocathiacins used in this study are enantiomerically diastereomerically pure and have up to 10 chiral centers. The lack of diastereoselectivity could be due to the fact that the reaction site is far away from any of the chiral centers. As a result, the chiral centers have little or no influence on the stereochemical outcome of the addition.

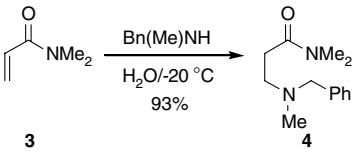
Michael addition of hetero-nucleophiles in frozen water was not limited to nocathiacins. At −20 °C, amines (10 equiv) and thiols (3 or 10 equiv) very efficiently added to simple substrates such as dehydroalanine amide **1**[12,15a] and *N*,*N*-dimethylacrylamide **3**[15b] to provide the corresponding Michael adducts in very good to excellent yields (Schemes 2 and 3). The addition of dimethylamine to **1** was complete in 3.5 h and furnished quantitative yields of **2a**. On the other hand, addition of 2-(*N*-methylamino)ethanol and morpholine to **1** required 7 and 9 days, respectively, for completion, but still provided the respective adducts **2b** and **2c** in very high



1 **2**

2a: XR" = NMe_2; 100%
2b: XR" = $N(Me)CH_2CH_2OH$; 90%
2c: XR" = $N(CH_2CH_2)_2O$; 90%
2d: XR" = $SCH_2CH_2NMe_2$; 95%
2e: XR" = $SCHMe_2$; 80-85%

Scheme 2.



Scheme 3.

yields. Addition of the sulfur nucleophile 2-(*N*,*N*-dimethylamino)ethanethiol (3 equiv) to **1** was complete in 3 h and provided the desired product **2d** in 95% isolated yields. However, addition of 3 equiv of 2-propanethiol was slow and required 4 days for completion, whereas with 10 equiv of 2-propanethiol, the reaction was complete in 24 h to furnish the adduct **2e** in very good yields. Also, under these conditions, *N*-benzylmethylamine underwent Michael addition to **3** (5 equiv) and provided the adduct **4** in 93% yield after 24 h.

In conclusion, we have described the first Michael addition in frozen water and application of this efficient methodology to modification of the functionally complex nocathiacins as well as simpler substrates. Most importantly, we discovered that the reaction in frozen water increased chemoselectivity and minimized unwanted side reactions, providing cleaner reaction mixtures, and higher isolated yields of Michael adducts. Finally, this unique phenomenon merits detailed mechanistic study.

Acknowledgements

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3.22 (2H, br s), 3.12 (1H, br s), 2.89 (7H, m), 2.47 (1H, br s), 2.13 (1H, m), 2.00 (3H, s), 1.93 (1H, d, $J = 14.7$ Hz), 1.61 (3H, s), 1.15 (3H, b s), 0.81 (3H, d, $J = 7.0$ Hz). LRMS (ESI) calcd for $C_{65}H_{70}N_{15}O_{19}S_5$ (M+H): 1524.4; found: 1524.5. Anal. Calcd for $C_{65}H_{69}N_{15}O_{19}S_5 \bullet 2.5$ HCl $\bullet 5.8$ H_2O: C, 45.38; H, 4.87; N, 12.21; S, 9.32; Cl, 5.15; found: C, 45.11; H, 4.71; N, 12.15; S, 9.25; Cl, 5.19.

15. (a) Reactions were run using 0.1 mmol of **1** in 1 mL of de-ionized water; (b) The reaction was run using 5 mmol of **3** in 10 mL of de-ionozed water.