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Reaction of cryptophycin 52 with thiols

Michael J. Martinelli,* Rajappa Vaidyanathan, Vien Van Khau and Michael A. Staszak

Chemical Process R&D, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285-4813, USA

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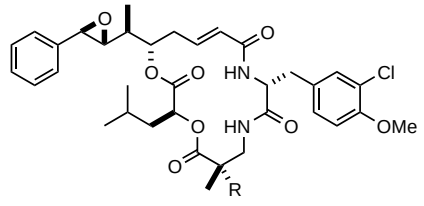
Abstract—The chemical reactivity of cryptophycin 52 towards sulfur and phosporous nucleophiles under different conditions was explored to reveal the differential reactivity of the epoxide and olefinic centers. Under basic conditions 1,4-addition to the enone is favored, while under acidic conditions the epoxide is more reactive. © 2002 Published by Elsevier Science Ltd.

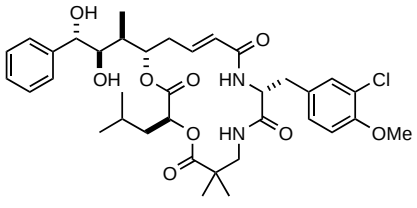
The cryptophycins are a class of tumor-selective cytotoxins isolated from cyanobacteria of the genus *Nostoc*.[1] Moore and co-workers isolated a host of cyclic depsipeptides including cryptophycin A, **1**, from the GSV 224 strain, and established their absolute configuration.[2] Cryptophycin A was found to be a potent antifungal agent, especially effective against *Cryptococcus*, a pathogen that infects immunodeficient persons. Cryptophycin A exhibited an IC_{50} value of 5 pg/mL against KB cells. In addition, **1** showed excellent activity against a broad spectrum of solid tumors, especially the drug-resistant phenotypes.[3]

Subsequent to the first total synthesis of cryptophycin A by Tius et al.,[4] several syntheses have been reported.[5] A number of synthetic cryptophycins, including cryptophycin 52 (**2**) have been prepared. Preliminary animal data suggest that **1** and **2** may be useful against breast, pancreatic and colon tumors. Cryptophycin A binds tightly to tubulin, and inhibits microtubule assembly and nucleotide exchange on tubulin. Panda and co-workers studied the mechanism of action of cryptophycin, and suggested that the antiproliferative and antimitotic activity is due to its reversible binding to the ends of microtubules, resulting in the most potent suppression of microtubules described to date.[6] It has also been shown that these compounds bind to the Vinca binding domain on tubulin, or to a site that overlaps with the Vinca site, and not the taxol domain.

Pre-clinical metabolism and solution stability studies revealed the reactivity of the epoxide towards H_2O



1 R=H Cryptophycin A
2 R=CH_3 Cryptophycin 52



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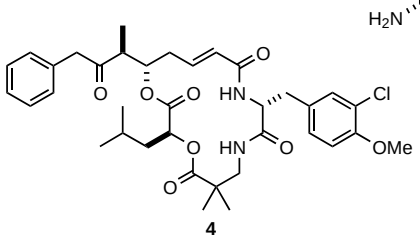

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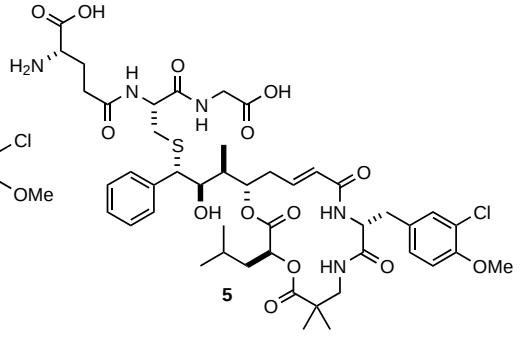

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* Corresponding author.

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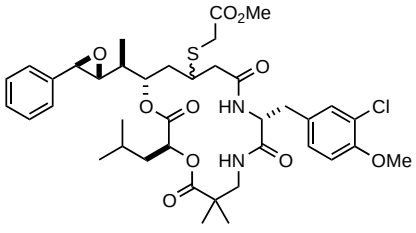
addition, leading to diol **3**.[7] Clinical trials, however, suggest that cryptophycin 52 (**1**) is secreted as the glutathione conjugate **5**, as a major metabolite. Further metabolism studies revealed that the metabolite isolated from rat and mouse plasma is the glutathione adduct **5** arising out of ring opening of the epoxide functionality. We were interested in gaining chemical insight into the reactivity site and mechanism of glutathione conjugation to cryptophycin 52.

Our initial attempts to incubate cryptophycin 52 with glutathione (with or without glutathione S-transferase) were unsuccessful, mainly due to the low solubility of cryptophycin in aqueous media. Therefore, we chose to use methyl thioglycolate as a glutathione mimic to study the reactivity profile of cryptophycin 52 with thiols. The epoxide and the α,β-unsaturated carbonyl moieties represent the most electrophilic sites in this molecule. The two lactone carbonyls might also be susceptible to nucleophilic addition, but are flanked with extensive substitution, affording enhanced stability. The reaction of cryptophycin 52 with methyl thioglycolate could conceivably furnish three adducts, **6**, **7** and **8**.[8]



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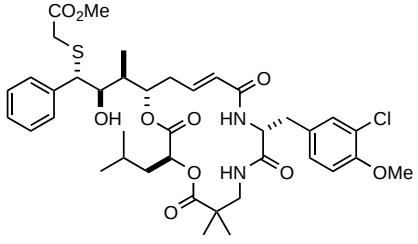

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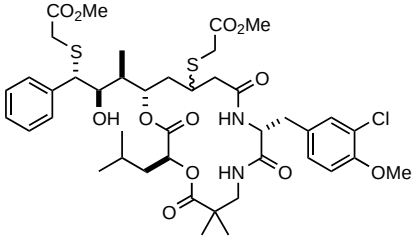

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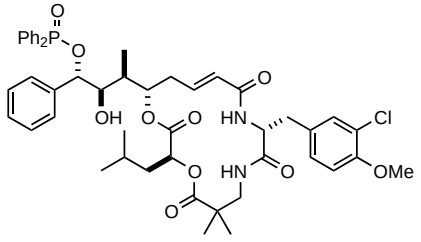
Cryptophycin 52 was treated with methyl thioglycolate under both acidic and basic conditions. Our results are summarized in Table 1. Treatment of cryptophycin 52 with 20 equiv. each of methyl thioglycolate and Et_3N for 3 days led to the formation of Michael adduct **6** and the bis adduct **8** in a 2.2:1 ratio (entry 1). Longer reaction times resulted in the formation of the bis adduct exclusively (entry 2). These results were in contrast to our metabolism studies, where the epoxide-opening product **3** was the only product observed.[7] We presumed that the 1,4 adduct formation would be reversible, while epoxide adduct formation would be irreversible. If this were to be the case, incubation of **2** with 1 equiv. of $HSCH_2CO_2Me$ and an excess of base should afford the epoxide-opening product **7**. However, our experiments indicated that the Michael adduct **6** was the sole product isolated under these conditions (entry 3). Performing the reaction in the absence of base did not lead to any of the methyl thioglycolate adducts. Instead, only diol **3**, arising presumably out of adventitious hydrolysis of the epoxide, was isolated (entry 4). Aqueous buffers were next examined. Using pH 7.4 buffer, a 4.2:1.3:1 ratio of **6**:**7**:**8** was observed in an overall yield of 39% (entry 6). Shorter reaction times did not lead to any reaction (entry 5). More basic conditions (pH 10 buffer) furnished the products in a 5.6:1:2.6 ratio in 84% yield (entry 7). After having examined the reaction of cryptophycin 52 with methyl thioglycolate under basic conditions, we sought to probe the outcome of the reaction in the presence of a Lewis acid. Accordingly, **2** was treated with 1 equiv. each of methyl thioglycolate and $BF_3{\cdot}OEt_2$. In contrast to the basic conditions described above, this reaction afforded a 1.5:1 mixture of ketone **4** and the epoxide-opening product **7** (entry 8). Neither of the other two adducts **6** nor **8** could be detected by LCMS. We envisioned that we could suppress the Lewis acid-mediated pinacol rearrangement of the epoxide to ketone **4** under the reaction conditions by using an excess of the sulfur nucleophile. Thus, when **2** was treated with 10 equiv. of $HSCH_2CO_2Me$ and 1 equiv. of $BF_3{\cdot}OEt_2$, **7** and **4** were obtained in a 3:1 ratio, and an isolated yield of 54% for adduct **7** (entry 9).

Table 1.

Entry	Equiv. $HSCH_2CO_2Me$	Conditions	**6**:**7**:**8**	% Yield
1	20	Et_3N (20 equiv.), THF/DMSO (1:4), rt, 3 days	2.2:0:1	89
2	10	Et_3N (10 equiv.), THF/MeOH (1:1), rt, 11 days	0:0:1	91
3	1.2	Et_3N (10 equiv.), THF/MeOH (1:1), rt, 8 days	1:0:0	70
4	10	THF/DMSO (1:4), rt, 12 days	NA[a]	90[a]
5	3	pH 7.4 buffer, THF, rt, 24 h	NA[b]	
6	10	pH 7.4 buffer, THF, rt, 8 days	4.2:1.3:1[c]	39
7	10	pH 10 buffer, THF, rt, 5 days	5.6:1:2.6	84
8	1	$BF_3{\cdot}OEt_2$ (1 equiv.), CH_2Cl_2, 16 h	0:1:0[c,d]	
9	10	$BF_3{\cdot}OEt_2$ (1 equiv.), CH_2Cl_2, 16 h	0:1:0[c,d]	54

[a] Only diol **3**, arising out of hydrolysis of the epoxide was obtained.
[b] Cryptophycin 52 was the only compound isolated.
[c] Ratios by LCMS.
[d] LCMS showed ketone **7** also; see text.

During the course of our synthetic efforts involving a macrocyclization protocol employing diphenylphosphinic chloride, we uncovered a rather facile reaction of diphenylphosphinic acid with cryptophycin 52. Heating a crude toluene mixture of cryptophycin 52 with this acid resulted in complete ring opening of the epoxide to form adduct **9** as a single diastereomer. It is also of interest to note that no conjugate addition was observed under these conditions. This observation is interesting to note in the context of potential in vivo nucleophiles that could react covalently with cryptophycin 52.



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In summary, our studies have revealed the reactivity profile of cryptophycin 52 with sulfur nucleophiles under different conditions. Under basic conditions, the preferred site of nucleophilic attack seems to be the enone functionality. In the presence of excess nucleophile, bis adduct formation is observed. The presence of Lewis acids leads to the nucleophilic ring opening of the epoxide. These results may provide further insight into the reactivity of this novel class of oncolytics under physiological conditions. Extrapolation to other oncolytic olefinic compounds may also be possible.

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8. New compounds were fully characterized and had the following physical properties:
   Michael adduct 6: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J=9.0 Hz, 1H), 7.38–7.30 (m, 4H), 7.25 (dd, J=1.5, 8.0 Hz, 2H), 7.16 (dd, J=2.0, 8.5 Hz, 1H), 6.79 (d, J=8.5 Hz, 1H), 6.74 (dd, J=5.0, 8.5 Hz, 1H), 4.96 (dd, J=4.5, 11.0 Hz, 1H), 4.83 (app dt, J=3.0, 10 Hz, 2H), 3.83 (s, 3H), 3.73 (s, 3H), 3.69–3.62 (m, 2H), 3.35 (dd, J=4.0, 14.0 Hz, 1H), 3.31 (s, 2H), 3.23 (dd, J=5.0 Hz, 13.0 Hz, 1H), 3.03 (dd, J=10.0, 14.0 Hz, 1H), 2.85 (dd, J=2.0, 7.5 Hz, 1H), 2.77 (d, J=17.5 Hz, 1H), 2.69 (app t, J=10.5 Hz, 1H), 2.16–2.02 (m, 2H), 1.80–1.64 (m, 4H), 1.40–1.35 (m, 1H), 1.22 (s, 3H), 1.12 (s, 3H), 1.11 (d, J=8.5 Hz, 3H), 0.84 (d, J=6.5 Hz, 3H), 0.83 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.5, 172.7, 171.1, 170.2, 159.6, 136.7, 131.7, 131.1, 128.8, 128.7, 128.4, 125.6, 125.3, 121.7, 111.9, 74.9, 70.9, 62.9, 58.7, 56.0, 54.6, 53.0, 47.5, 44.1, 41.8, 40.4, 39.7, 39.1, 37.6, 35.6, 33.0, 24.7, 22.9, 22.6, 21.6, 21.3, 13.5.
   Bis adduct 8: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J=9.0 Hz, 1H), 7.96–7.30 (m, 6H), 7.18 (dd, J=2.0, 8.5 Hz, 1H), 6.81–6.76 (m, 2H), 4.93–4.90 (m, 2H), 4.84 (dt, J=3.0, 9.0 Hz, 1H), 4.00 (d, J=10.0 Hz, 1H), 3.97–3.95 (m, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.71–3.69 (m, 1H), 3.66 (s, 3H), 3.45 (d, J=18.0 Hz, 1H), 3.36 (d, J=18.0 Hz, 1H), 3.20 (dd, J=4.5, 13.5 Hz, 1H), 3.05 (dd, J=10.0, 14.0 Hz, 1H), 3.00 (d, J=15.5 Hz, 1H), 2.84 (d, J=15.5 Hz, 1H), 2.81 (d, J=18 Hz, 1H), 2.77–2.71 (m, 1H), 2.31–2.24 (m, 1H), 2.11 (dd, J=10.5, 18.0 Hz, 1H), 1.95–1.91 (m, 2H), 1.79–1.67 (m, 3H), 1.43–1.42 (m, 1H), 1.38 (br s, 1H), 1.23 (s, 3H), 1.13 (s, 3H), 1.03 (d, J=6.5 Hz, 3H), 0.93 (d, J=6.5 Hz, 3H), 0.89 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.4, 172.5, 171.2, 170.4, 170.3, 153.6, 138.0, 131.9, 131.3, 129.1, 128.9, 128.8, 128.6, 128.5, 121.8, 111.9, 74.9, 72.4, 71.0, 56.1, 54.5, 53.1, 52.9, 52.4, 47.7, 44.3, 41.2, 38.6, 38.3, 38.2, 37.7, 35.7, 33.2, 31.9, 24.8, 23.2, 22.7, 21.7, 21.5, 8.9.
   Epoxide opening adduct 7: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.31 (m, 5H), 7.22–7.21 (m, 2H), 7.07 (dd, J=2.0, 8.5 Hz, 1H), 6.85 (d, J=8.5 Hz, 1H), 6.78 (ddd, J=4.0, 10.5, 15.0 Hz, 1H), 5.76 (d, J=15.0 Hz, 1H), 5.52 (d, J=8.0 Hz, 1H), 5.15 (app t, J=9.5 Hz, 1H), 4.90 (dd, J=4.0, 9.5 Hz, 1H), 4.74–4.71 (m, 1H), 4.03 (d, J=9.5 Hz, 1H), 3.96–3.94 (m, 1H), 3.88 (s, 3H), 3.67 (s, 3H), 3.39 (dd, J=8.5, 13.5 Hz, 1H), 3.18–3.16 (m, 3H), 3.01 (d, J=15.5 Hz, 1H), 2.85 (d, J=15.5 Hz, 1H), 2.38–2.26 (m, 2H), 1.79–1.66 (m, 2H), 1.44–1.37 (m, 2H), 1.23 (s, 3H), 1.17 (s, 3H), 1.06 (d, J=7.0 Hz, 3H), 0.92 (d, J=6.5 Hz, 3H), 0.91 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.1, 170.7, 170.6, 170.5, 165.3, 154.3, 142.9, 138.4, 131.2, 129.8, 129.3, 129.2, 128.7, 128.5, 124.6, 122.8, 112.5, 76.1, 72.9, 71.6, 56.4, 54.6, 53.1, 52.7, 46.7, 43.0, 39.8, 39.7, 36.8, 35.6, 29.9, 25.0, 23.3, 23.1, 22.9, 21.8, 9.0.