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Synthesis of Tetrocarcin Derivatives with Specific Inhibitory Activity Towards Bcl-2 Functions

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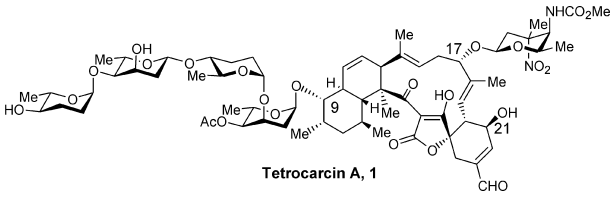
Abstract—Tetrocarcin A was recently identified as an inhibitor of the anti-apoptotic function of Bcl-2. We synthesized novel tetrocarcin derivatives in order to increase their selective inhibitory activity against Bcl-2. It was found that 21-acetoxy-9-glycosyloxy derivatives had potent Bcl-2 inhibitory activity without significant antimicrobial activity.

Introduction

Bcl-2 is a membrane protein that inhibits apoptosis induced by various stimuli.[1] Elevated expression of Bcl-2 is often found in human cancers. Consequently, those cancer cells resist apoptosis.[2] In many cases, cancers that overexpress Bcl-2, such as follicular lymphoma and hormone-refractory prostate cancer, are also resistant to chemotherapeutic agents. Thus, inhibitors of anti-apoptotic function of Bcl-2[3] are potentially useful in treating human malignancies, particularly those that overexpress Bcl-2. We have recently reported tetrocarcin A (**1**) as the first small molecule inhibitor of the anti-apoptotic function of Bcl-2.[4] Compound **1** induced apoptosis in cell lines that overexpressed Bcl-2 and were resistant to cell death stimuli, such as those induced by anti-Fas antibody (αFas), tumor necrosis factor α or staurosporine. Although **1** was originally discovered as an antibiotic that was active against Gram-positive bacteria,[5] the absence of Bcl-2 family proteins in bacteria suggests that its antibacterial activities are not related to its activity against Bcl-2. We synthesized tetrocarcin derivatives in order to achieve potent Bcl-2 inhibitory activity without antibacterial activities. Here we report the synthesis and biological evaluation of tetrocarcin derivatives.



Tetrocarcin A, 1

Chemistry

Monomethyl ether of the tetronic acid moiety **2** was synthesized by treatment of **1** with trimethylsilyl (TMS) diazomethane.[6] In order to introduce various substituents to the 9 position of **1**, the original sugar chain was removed by hydrolysis under acidic conditions.[5d] Compound **3**, which has hydroxy groups at the 9 and 21 positions, was converted to disilyloxy derivative **4** by treatment of 10 equiv of *tert*-butyldimethylsilyl chloride (TBDMSCl) for 6 days and only the silyl group at the 21 position was removed selectively with tetrabutylammonium fluoride (TBAF). On the other hand, 21-monosilyloxy derivative **6** was obtained by treatment with 5 equiv of TBDMSCl for 2 days in 77% yield. Different kinds of modifications at the 9 and 21 positions can be achieved by using **5** and **6** as starting materials. For example, **6** was condensed with carboxylic acids in the presence of 1-(3-dimethylamino)propyl-3-ethylcarbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP), followed by removal of the silyl group to give 9-acyloxy derivatives, such as **8** (Scheme 1). We also synthesized derivatives with substituents at the 9 and 21 positions from 9-hydroxy-21-acetoxy derivative **9** as

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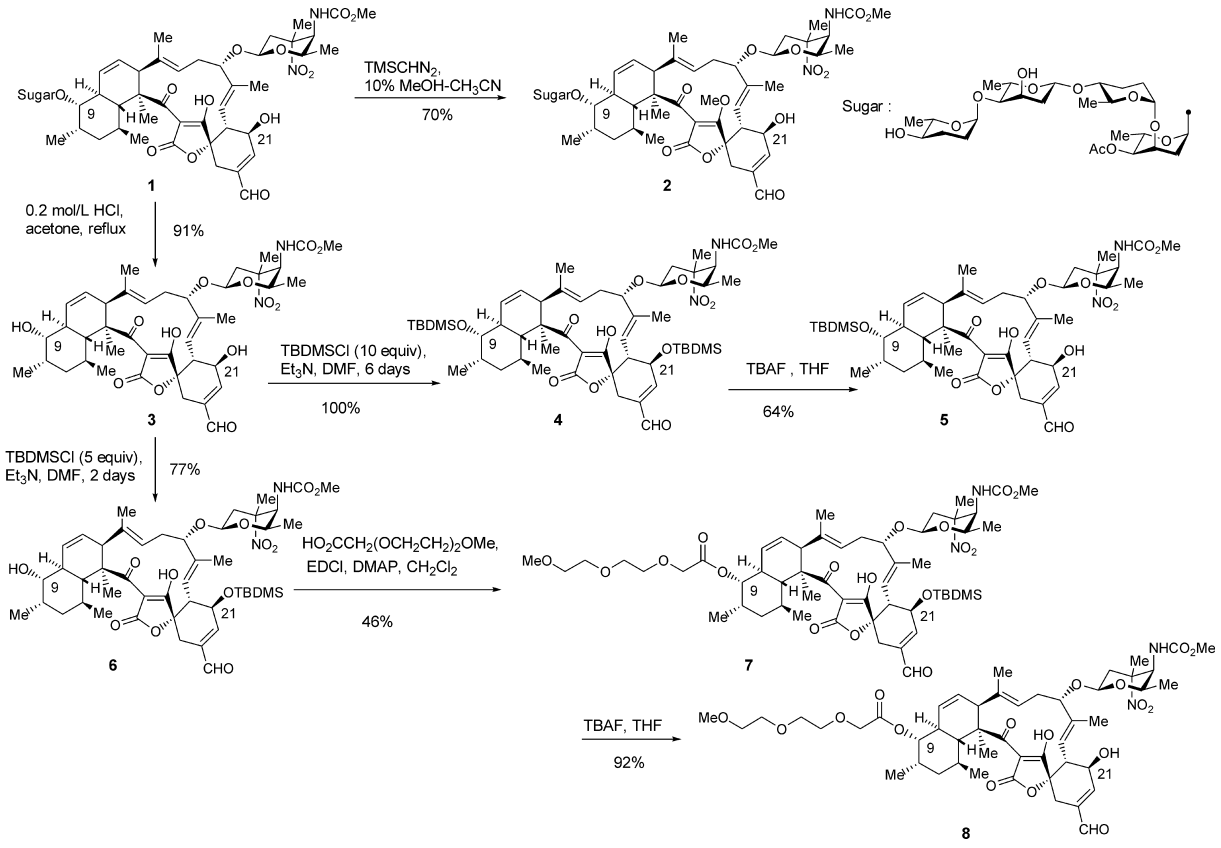
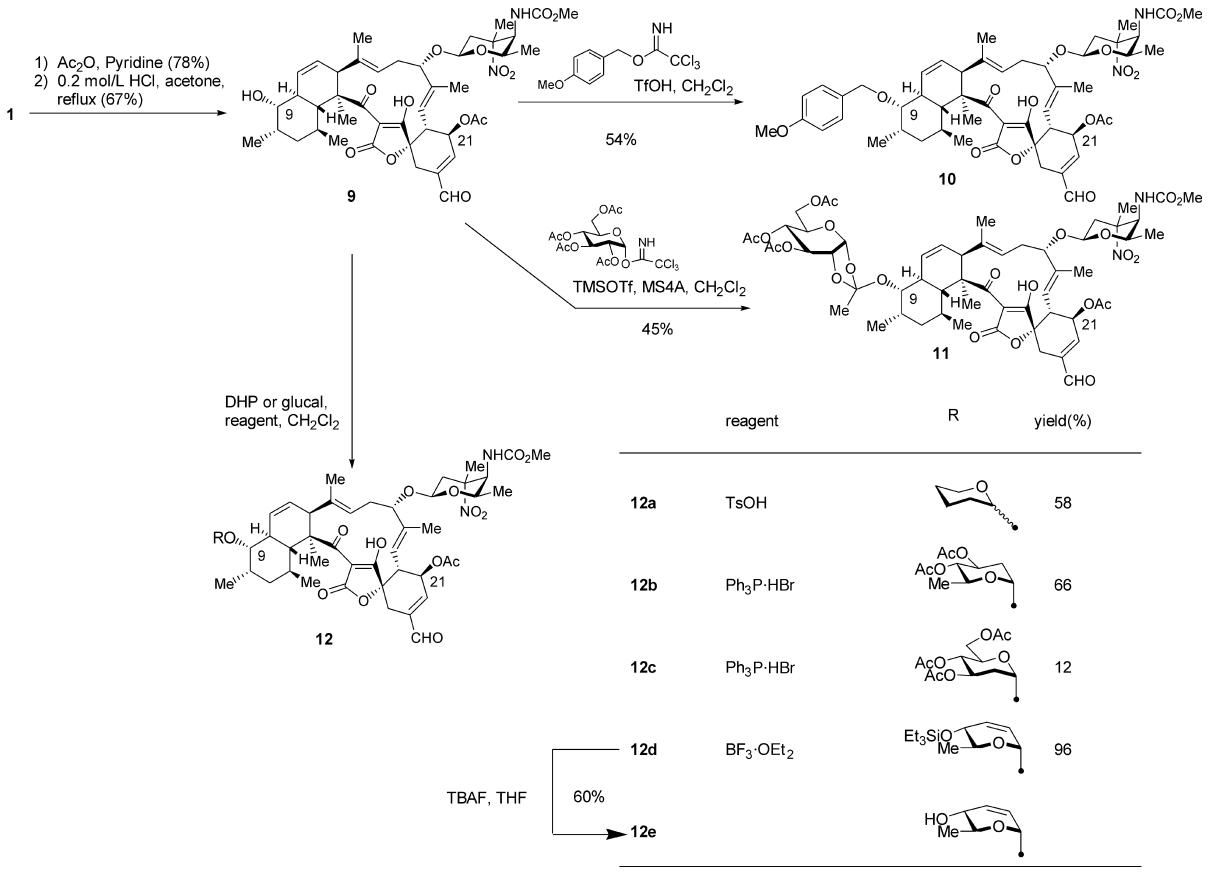


Scheme 1.

shown in Scheme 2. Compound **9** was synthesized from the peracetate of **1** by hydrolysis under the same conditions as synthesis of **3**.[5d] Compound **10** was synthesized from **9** and 4-methoxybenzyl 2,2,2-trichloroacetimidate in the presence of trifluoromethanesulfonic acid (TfOH).[7] By the reaction of **9** with tetra-*O*-acetylglucosyl 2,2,2-trichloroacetimidate and trimethylsilyl trifluoromethanesulfonate (TMSOTf), orthoester **11** was obtained in 45% yield. Tetrahydropyranyl (THP) ether **12a**, the simplest analogue of the original sugar, and deoxyglycosides **12b** and **12c**, could be prepared from **9** and 3,4-dihydro-2*H*-pyran (DHP) or corresponding glucals. THP ether **12a** was a mixture of diastereomers. Compound **12b** and **12c** were obtained stereoselectively using $Ph_3P{\cdot}HBr$ without Ferrier rearrangement.[8] Ferrier rearrangement product **12d** was synthesized by using $BF_3{\cdot}OEt_2$ in good yield. Compound **12d** was converted to **12e** by treatment with TBAF.

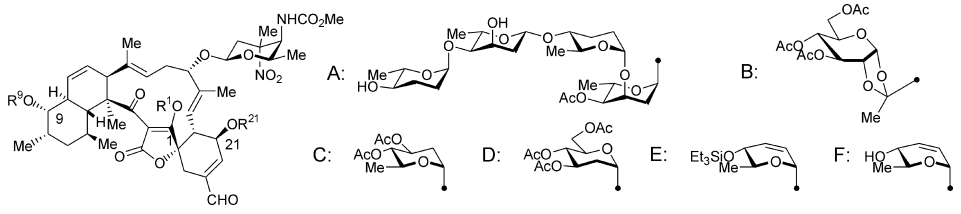
Biological Evaluations

Bcl-2 overexpressing HeLa cells (HeLa/bcl-2) were used for studying the Bcl-2 inhibitory activities of tetrocarin derivatives.[4] We have previously determined that activation of caspases is a quantitative indicator for apoptosis induction associated with Bcl-2 inhibition by **1**. Caspase assay (DEVDase assay) using a tetrapeptide substrate, Ac-DEVD-MCA [acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid α-(4-methyl-coumaryl-7-amide)], was used for SAR analysis of Bcl-2 inhibition (Table 1). Addition of αFas to HeLa/bcl-2 cells showed no caspase activation, but co-treatment of αFas-resistant HeLa/bcl-2 cells with **1** and αFas caused DEVDase activation. The concentration of **1** needed for αFas-dependent 3-fold activation of DEVDase (AC_3) was 2.2 μM. In the absence of αFas, **1** weakly activated DEVDase in an αFas-independent manner with the AC_3 of 7.7 μM, indicating that **1** itself retained weak activity as an apoptotic stimulus. Although methyl ether **2** showed more potent Fas-dependent DEVDase activity than **1**, its Fas-independent DEVDase activity was also increased with AC_3 of 2.5 μM. One class of non-sugar type derivatives, such as **3**, **4**, **6** and **8**, lost the DEVDase activity both in the presence and absence of αFas. The other non-sugar type of derivatives, such as **5** and **10**, showed αFas-dependent DEVDase activity but their αFas-independent DEVDase activities were increased. Deoxy sugar derivatives **12b–e** showed no αFas-independent DEVDase activity but still retained αFas-dependent DEVDase activity, indicating that they did not induce apoptosis by themselves but instead inhibited anti-apoptotic function of Bcl-2. αFas-dependent DEVDase activity of **12b** (KF67544) was almost comparable to **1**. The SAR obtained for DEVDase activation was very similar to that obtained from XTT cell viability assay. It is interesting to note that these derivatives showed very weak antibacterial activities. Thus, the introduction of deoxy sugar at the 9 position was effective for selective Bcl-2 inhibitory activity, but had no effect on other Bcl-2-unrelated properties, such as antimicrobial activities and apoptosis inducing activities.



Scheme 2.

Table 1. Biological activities of tetrocarcin derivatives



Compds				DEVDase assay[a] AC_3 (μM)		XTT assay[b] IC_{50} (μM)		BS[c] MIC (μg/mL)
	R^1	R^9	R^{21}	Fas+	Fas−	Fas+	Fas−	
1	H	A	H	2.2	7.7	1.4	4.0	<0.049
2	Me	A	H	1	2.5	0.8	1.5	5.2
3	H	H	H	>10	>10	>10	>10	>50
4	H	TBDMS	TBDMS	>10	>10	>10	>10	>50
5	H	TBDMS	H	1.3	1.8	2.0	2.0	>50
6	H	H	TBDMS	>10	>10	>10	>10	>50
7	H	$MeO(CH_2CH_2O)_2CH_2CO$	TBDMS	9.5	>10	3.9	6.4	>50
8	H	$MeO(CH_2CH_2O)_2CH_2CO$	H	>10	>10	>10	>10	>50
9	H	H	Ac	8.4	>10	>10	>10	>50
10	H	*o*-$MeOC_6H_5CH_2$	Ac	3.8	4.8	7.6	5.8	>50
11	H	B	Ac	5.7	>10	1.8	>10	>50
12a	H	THP	Ac	3.9	4.9	3.9	4.8	6.3
12b	H	C	Ac	2.1	>10	1.1	>10	16.7
12c	H	D	Ac	3.0	>10	2.0	>10	>50
12d	H	E	Ac	4.9	>10	4.3	>10	>50
12e	H	F	Ac	3.6	>10	3.7	>10	>50

[a]The effect of compds on DEVD cleavage with (+) or without (-) anti-Fas antibody treatment. (AC_3 = concentration of 3-fold activation).
[b]XTT assay is for evaluation of cell viability with (+) or without (-) anti-Fas antibody treatment (ref. 4).
[c]Antimicrobial activity against *Bacillus subtilis*.

Conclusion

From a SAR study of tetrocarcin derivatives, it was clear that the sugar moiety at the 9 position played an important role in Bcl-2 inhibitory activity. Among the sugar derivatives of **1**, a series of deoxy sugar analogues was found to retain Bcl-2 inhibitory activity but lost both apoptosis inducing activity and antimicrobial activity. Combined use of this class of tetrocarcin derivatives with clinically useful anticancer agents might be beneficial for treating chemotherapy-resistant human tumors that overexpress Bcl-2.

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