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Fluorination of triptolide and its analogues and their cytotoxicity

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Abstract—The reaction of triptolide and its analogues with a fluorinating agent, that is, bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor) or (diethylamino)sulfur trifluoride (DAST), was studied. One of the fluorinated products, 14β-dehydroxy-14β-fluoro triptolide, was found to be more cytotoxic than the parent natural triptolide.

Fluorination of biologically active natural products is known to sometimes enhance the activity. Therefore, in the medicinal chemistry, fluorination of such compounds is often designed and studied[1] by using dimethylaminosulfur trifluoride (DAST) or bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor), well-known efficient nucleophilic fluorinating reagents for hydroxyl and carbonyl groups.[2] Of triptolide (**1a**) and its related compounds (**2**–**4**) isolated from *Tripterygium wilfordii* (Celastaceae) (Fig. 1), **1a**, **2**, and **3** have a unique triepoxide system on the B/C ring system, which is reported to be associated with the cytotoxic activity.[3,4] In our previous paper on the synthesis of triptolide analogues,[5] we reported that the stereochemistry at C-14 and the presence of 12α, 13α-oriented epoxide might be important and essential factors for the triptolide analogues to show cytotoxic activity. This present paper describes the reactions of natural triptolide and its analogues with these fluorinated agents and the cytotoxic activities of the products on human tumor cells.

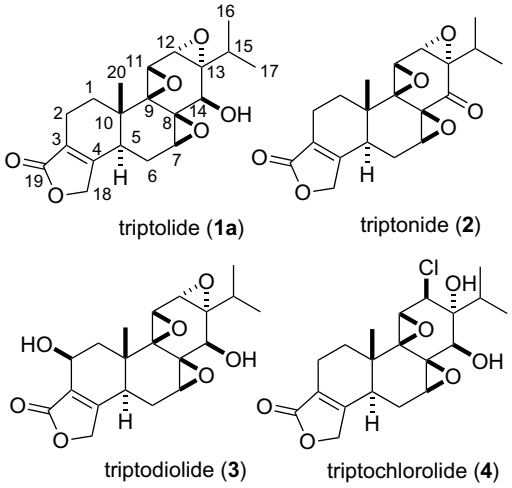

Figure 1. Triptolide (**1a**) and its related compounds (**2**–**4**) from *Tripterygium wilfordii*.

Starting material **1a** was isolated from *T. wilfordii*, and the other starting materials, 14-*epi*-triptolide (**1b**) and 12α-hydroxytriptolide (**8**), were prepared from **1a** according to the reported procedure.[4] Compound **2** was also prepared for comparison by the procedure described.[5] Fluorination of triptolide (**1a**) proceeded efficiently to give the corresponding 14α-fluorinated analogue (**5a**) in 77% yield, as reported in Ref. 6 (Scheme 1).

On the other hand, fluorination of 14-*epi*-triptolide (**1b**) gave the corresponding 14β-oriented fluorinated compound (**5b**) in very poor yields along with a series of by-products. The reactions of **1b** with DAST or Deoxo-Fluor under several different reaction conditions are summarized in Table 1. When the reaction was car-

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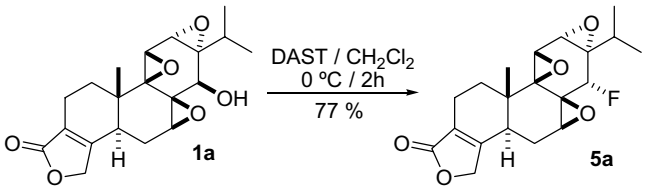


Scheme 1. Reaction of triptolide (**1a**) with DAST.

ried out with DAST at 0 °C for 3 h, for example, the corresponding 14β-fluorinated compound **5b** was obtained in a yield of 12%, along with **6**, **7**, **5a**, and the starting material **1b** in yields of 28%, 20%, 6%, and 2%, respectively (entry 2 in Table 1). The addition of bases[7] apparently had no effect (entries 6–8 in Table 1). The difference in the reactivity was not observed between the two fluorinating agents, DAST and Deoxo-Fluor (entries 3 and 9 in Table 1). The structures of the products were determined on the basis of ^{1}H and ^{13}C NMR, of which those of **5a**, **b**, and **6** were further confirmed by the X-ray crystallographic analysis. The ORTEP representation of **6** is shown in Figure 2.[8]

The plausible reaction mechanism involved in the reactions producing **5a**, **b**, **6**, and **7** from **1b** is shown in Scheme 2. Thus, by the reaction with DAST, **1b** gave a carbocation **B** via the corresponding intermediate **A**. Some of the carbocation **B** gave, by further skeletal rearrangement, an intermediate **C**. By a nucleophilic attack

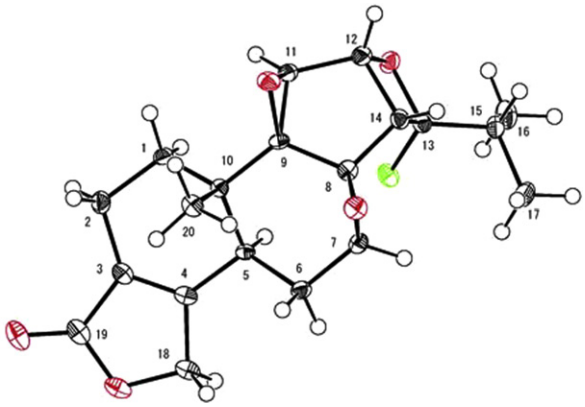
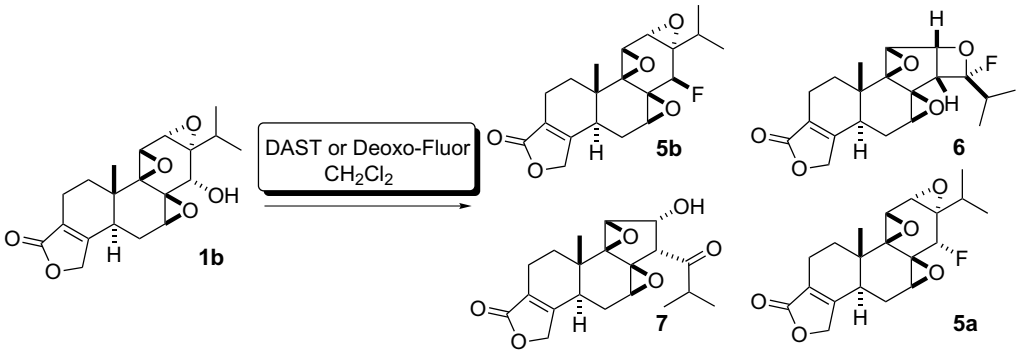


Figure 2. ORTEP representation of compound **6**.

by a fluoride anion, **B** gave 14α- and β-fluoro triptolide analogues **5a** and **b**, whereas **C** gave **6**. When the reaction mixture was treated with water after the reaction, **7** was considered to have been produced from the remaining **C** via hemi-acetal **D** (Scheme 2). Rearrangement of bicyclic α-oxiranyl cyclohexanol to the oxabicyclo[3.2.0] system is to be reported for the first time in this paper, though a skeletal rearrangement of bicyclic α-oxiranyl cyclooctanol to oxabicyclo[5.2.0] system with DAST is known.[9]

Fluorination of **8** gave, as shown in Scheme 3, α- and β-fluorinated triptolide analogues (**9a** and **9b**) in 11% and

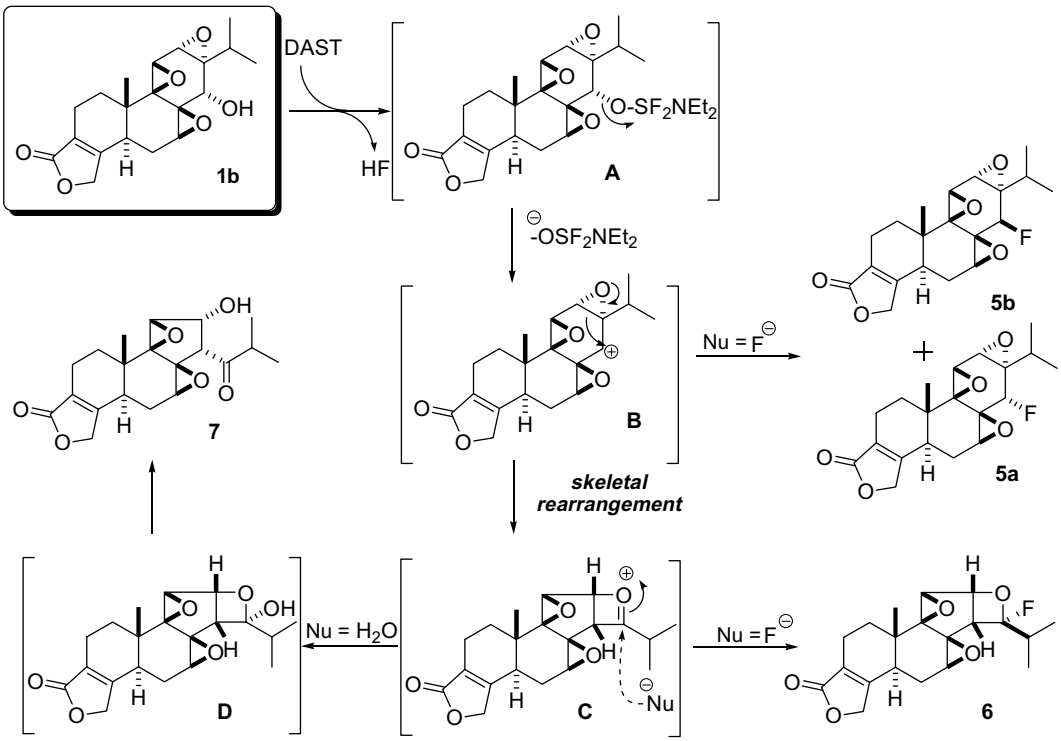
Table 1. Reactions of 14-*epi*-triptolide (**1b**) with fluorinating agents under several reaction conditions



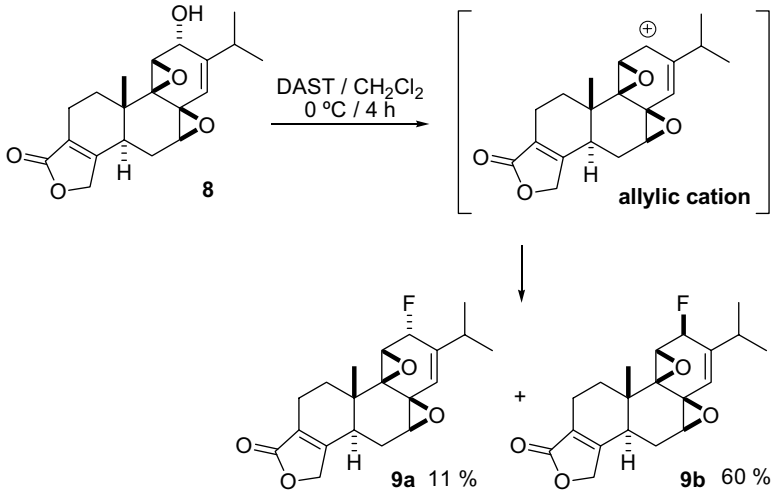
Entries	Reaction conditions[a]				Yields[b] (%)				
	Fluorinating agents (equiv)	Additives (equiv)	Reaction temp. (°C)	Reaction time (h)	**5b**	**6**	**7**	**5a**	**1b**
1	DAST (5)	None	−78	3	—	—	—	—	89
2	DAST (5)	None	0	3	12	28	20	6	2
3	DAST (5)	None	0	4	12	32	32	7	—
4	DAST (5)	None	rt	4	13	35	35	6	3
5	DAST (10)	None	0	4	10	23	28	6	2
6	DAST (5)	Pyridine (10)	0	4	—	—	—	—	62
7	DAST (5)	Et_3N (10)	0	4	—	—	—	—	88
8	DAST (5)	Et_3N (5)	60	4	—	—	—	—	85
9	Deoxo-Fluor (5)	None	0	4	11	32	32	10	2
10	Deoxo-Fluor (5)	None	rt	4	10	23	27	6	4

[a] The reaction was performed by adding a fluorinating reagent to a CH_2Cl_2 solution of **1b**. After the reaction, the reaction mixture was treated with AcOEt, washed with water, and evaporated to give a crude reaction product, which was separated by HPLC (column RP-18; eluting solvent MeCN:H_2O (45:55); detection by UV 215 nm).

[b] Yield after HPLC separation.



Scheme 2. Plausible reaction mechanism.



Scheme 3. Reaction of compound **8** with DAST.

60% yields, respectively. The results suggested that the reaction likely proceeded via the allylic cation.

Natural diterpene triptolide (**1a** and **2**) and its semisynthetic analogues prepared in the present study (**1b**, **5a**, **b**, **6**–**8**, **9a**, and **b**) were evaluated for their cytotoxic activities on A549 human lung and HT29 human colon tumor cells.

The results are shown in Table 2. Of them, 14β-fluoro triptolide analogue (**5b**) was found to be the most active and significantly more active than the parent natural triptolide (**1a**). The semisynthetic analogues **6** and **7** having five-membered C ring and analogues **8**, **9a**, and **b** with no 12,13-epoxide substructure and no fluoride atom were shown to be only weakly cytotoxic.

The results suggest that the electron-withdrawing group such as the fluoride atom on 14-carbon may enhance the cytotoxicity and that the stereochemistry of 14-carbon is important, as **1a** and **5b**, having 14β-substituents, are more potent than the corresponding 14α-substituted triptolide analogues (**1b** and **5a**). The relationship between the 3D-structure and activity was studied by aligning the X-ray structures of **1a**, **1b**, **2**, **5a**, and **5b**,[8] having relatively potent cytotoxicity, by their common A/B ring system, by using SYBYL7.3 software.[10] The superimpo-

Table 2. Cytotoxicity of triptolide (**1a**) and its analogues (**1b**, **2**, **5a**, **5b**, **6**–**8**, **9a**, and **9b**) on A549 and HT29 cells

Compound	IC_{50} (ng/mL)		Compound	IC_{50} (ng/mL)	
	A549	HT29		A549	HT29
1a	1.3	0.1	**6**	997	72
1b	59	9.1	**7**	>1.0	86
2	3.5	<0.46	**8**	81	17
5a	3.0	0.24	**9a**	1500	150
5b	0.42	0.06	**9b**	2200	210

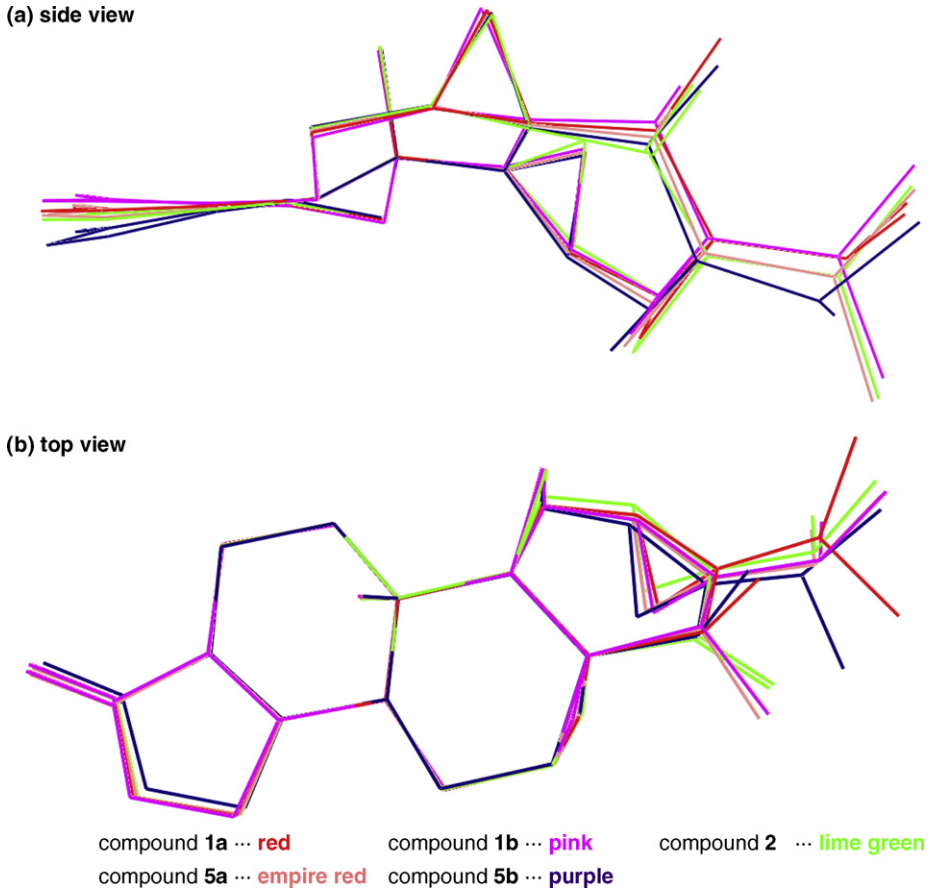


Figure 3. Stereodrawing of aligned compounds **1a**, **1b**, **2**, **5a**, and **5b** which were produced by X-ray crystallographic analysis.

sition drawings are shown in Figure 3. Although those of compounds **5b** and **1b**, having much higher activity and much weaker activity than **2**, respectively, were slightly deviated from those of **1a**, **2**, and **5a**, the 3D-whole molecule structures of **1a**, **1b**, **2**, **5a**, and **5b** were almost superimposable. The fact may suggest that a certain 3D-alignment is needed for the present series of triptolide analogues to be cytotoxic.

As described above, the reaction of 14-*epi*-triptolide (**1b**) with DAST or Deoxo-Fluor gave 14β-fluoro triptolide analogue (**5b**), which was much more cytotoxic than the natural and semisynthesized triptolide analogues known. The 3D-alignment of **5b**, revealed by X-ray crystallographic analysis, suggests that the conformation may be significantly related to the cytotoxic activity. New compounds **6** and **7**, having a novel carbon framework, were found to be produced by rearrangement in the present reaction.

Acknowledgments

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