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Absolute Configuration of C-1027 Chromophore

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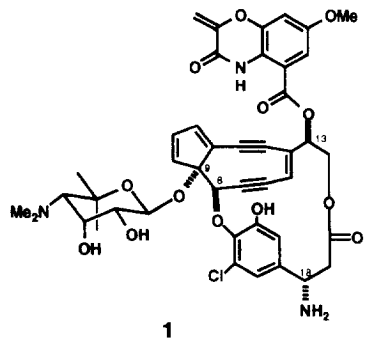
Abstract: The absolute configuration of C-1027 chromophore was determined as 8*R*, 9*R*, 13*R*, and 18*S* using the modified Mosher's, two-dimensional NMR, and molecular modeling methods.

Antibiotic C-1027, isolated from the broth filtrate of *Streptomyces globisporus* C-1027, is a new member of the chromoprotein antitumor antibiotics composed of an unstable enediyne chromophore and a carrier apoprotein.[2] Although the structures of C-1027 chromophore and its cycloaromatized product were elucidated,[3] the stereochemistry has been determined only for the aminosugar moiety.[4] Not only the absolute but the relative configuration of the core and macrolide moieties has not been revealed except for the trans relationship between C8 and C9.[3] In this communication we determined the stereostructures of C-1027 chromophore and the aromatized product to be **1** and **2**, respectively, by utilizing the modified Mosher's, two-dimensional (2D) NMR, and molecular modeling methods.[5]

Since the apoprotein-free C-1027 chromophore (**1**) is too labile, more stable **2** was used throughout this study. Attempts to derivatize **2** to a crystalline compound adequate for X-ray crystallography were unsuccessful. Therefore, we applied the modified Mosher's method[6] to determine the absolute configurations of C13 and C18. Chemical shift difference ($\Delta\delta$) of each proton between (*S*) and (*R*)-MTPA derivatives (**3**, **4**) listed in Figure 1 shows that the configurations of C18 and C13 are *S* and *R*, respectively. On the other hand, the 2D ROESY spectrum of **2** in DMSO-d_6 showed strong NOE between H5 and H13, but not between H3 and H13. The macrolide **2** is a fairly rigid molecule because it consists of two aromatic rings. Therefore, this NOE



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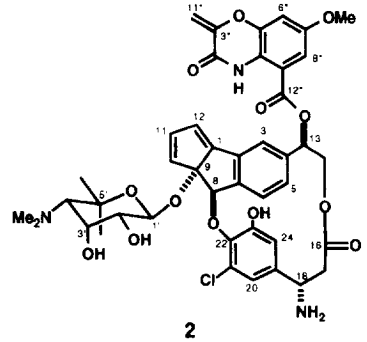

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strongly suggests that the configuration of C8 would be *R* (**5**) but not *S* (**6**) (Figure 2). Additional strong NOEs between H12 and the C23 phenol proton and between H18 and H24 clearly indicate that rotation of the benzene ring of the β-tyrosine moiety would be inhibited or restricted considerably, and that only the stereostructure of diastereomer **5** is in good agreement with those NOE data (Figure 2). Large chemical shift differences (Δδ) for H20 of **3** and H3 of **4** (Figure 1) suggest that those protons are located in close proximity to the MTPA groups, which would also support the structure **5**.

Thus, both configurations of C8 and C9 should be concluded to be *R*. This assignment was further supported by the intermolecular NOEs in a complex of **2** and apoprotein. An 8.6 mM solution of **2** and apoprotein in 10% D_2O/90% H_2O or 99.8% D_2O was adjusted to pH 5.0 with 0.3 M NaOD and 2D NMR measurements (COSY, HOHAHA, and NOESY, 30°C, Bruker AM 600)[7] was carried out for this complex (Ka = 1.5 x 10^4 M^{-1} by fluorescence quenching titration[8]). A total of 90% of the proton resonances of the apoprotein have been assigned by using the standard sequential assignment technique.[7,9] Complete assignment of the aromatized chromophore resonances and NOEs observed in the complex are listed in Table 1. Computer modeling of three-dimensional (3D) binding structure using a distance constraints based on the NOE data and a calculated apoprotein structure[10] was examined for the two possible isomers, **5** and **6**. Only **5** exhibited a reasonable binding structure without violation of the NOE distance constraints (Figure 3). However, no binding structure within the NOE bounds was able to be built for **6**.

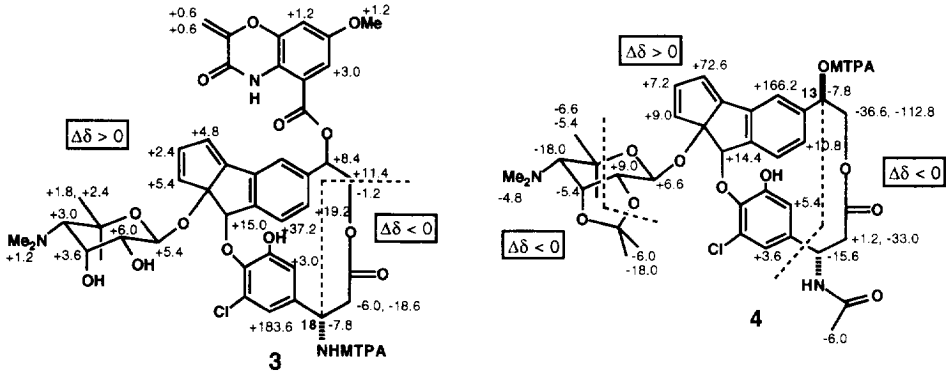

Figure 1. Δδ Values [(δ_S- δ_R) in hertz (600 MHz)] of MTPA amide (**3**) and MTPA ester (**4**).

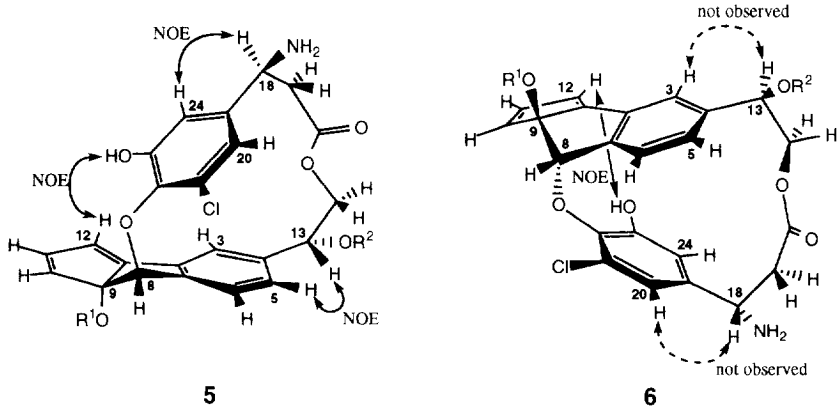

Figure 2. Stereostructures of the two possible stereoisomers, (**5**) [8*R*,9*R*] and (**6**) [8*S*,9*S*], for the aromatized chromophore.

Table 1. NOEs Observed in the Complex of **2** with Apoprotein (pH 5.0, 30°C).

		δ (ppm)[a]	Intramolecular NOEs	Intermolecular NOEs
Core	H3	6.98	H12(++)[b]; H14-1(++)[c]; H14-2(+)[c]	Tyr32 Cδ1,2H(+)[b]
	H5	7.02	H6(+++); H13(+++)	
	H6	7.31	H5(+++); H8(+++); H13(+)	Asn97 CαH(±)
	H8	5.67	H6(++); H1'(++)	
	H10	6.55	H8(+); H1'(±)	
	H11	6.74	H12(+++); H1'(±)	
	H12	6.40	H3(++); H11(+++); H1'(±)	
	H13	5.80	H5(+++); H6(+); H14-1(+)[c]; H14-2(++)[c]	Ile33 CαH(+); Ala34 NH(+)
	H14-1[c]	4.27	H3(++); H13(+)	
	H14-2[c]	4.46	H3(+); H13(+)	
	H17-1[c]	2.57	H18(+); H20(++); H24(+)	
	H17-2[c]	2.76	H18(+); H24(+)	
	H18	4.33	H17-1(++)[c]; H17-2(++)[c]; H24(+++)	
	H20	6.95	H17-1(++)[c]; H18(+)	
	H24	6.28	H17-1(+)[c]; H17-2(+)[c]; H18(++++)	
Aminosugar	H1'	3.90	H8(++); H10(±); H2'(+); 6'-CH_3α(++); H8"(+)	
	H2'	2.30	H1'(+); H3'(+++)	Tyr32 Cε1,2H(+)
	H3'	3.68	H2'(+++); H4'(++++)	
	H4'	2.58	H3'(++++); 6'-CH_3β(+++++)	Tyr32 Cε1,2H(++)
	4'-$N(CH_3)_2$	2.60	6'-CH_3α(+++)	
	6'-CH_3α	0.65	H1'(+++); 4'-$N(CH_3)_2$(+++)	
	6'-CH_3β	0.92	H4'(++++)	
Benzoxazine	1"-NH	10.21		Tyr32 Cβ-2H(+)[c]; Tyr32 Cδ1,2H(±)
	H6"	7.25		
	7"-OCH_3	3.48	H8"(+++++)	Pro76 Cβ-1H(++)[c]; Pro76 Cβ-2H(++)[c]
	H8"	7.11	H13(+); H1'(+); 7"-OCH_3(+++++)	Cys45 Cβ-1H(++)[c]; Pro76 CαH(++); Pro76 Cβ-1H(+)[c]; Pro76 Cβ-2H(++)[c]
	H11"-1[c]	5.32	H11"-2(++++)[c]	
	H11"-2[c]	5.58	H11"-1(++++)[c]	

[a] Referred to the water resonance at 4.71 ppm. [b] Relative intensities indicated in parenthesis. [c] Higher and lower-field resonances of diastereotopic protons indicated as -1 and -2, respectively.

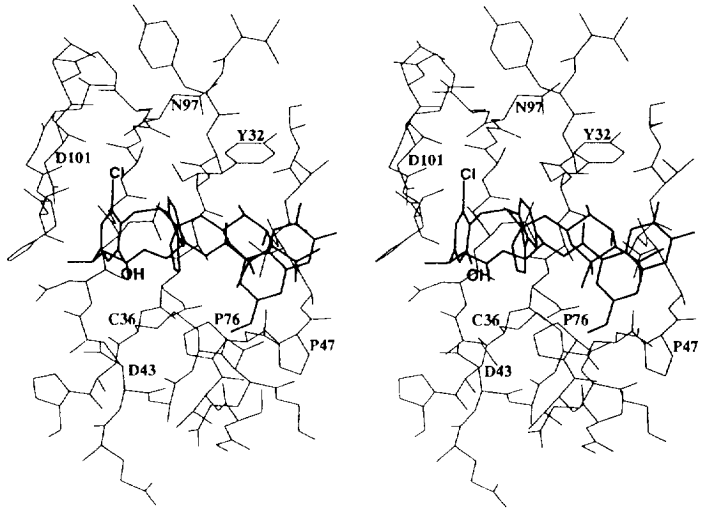

Figure 3. The NOE-restrained refined binding-structure of **5** (**2**) with apoprotein.

In summary, we have assigned the configuration of C-1027 chromophore **1** to be 8*R*, 9*R*, 13*R*, 18*S*. Noteworthy is that the C13 configuration is identical with that of the corresponding stereogenic center of kedarcidin chromophore[11] and is opposite to that of neocarzinostatin chromophore.[12] This disagreement may suggest a different biosynthetic origin of the C13 oxygen between the nine-membered enediyne (**1** and kedarcidin) and the epoxydiyne chromophore (neocarzinostatin).[13]

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