

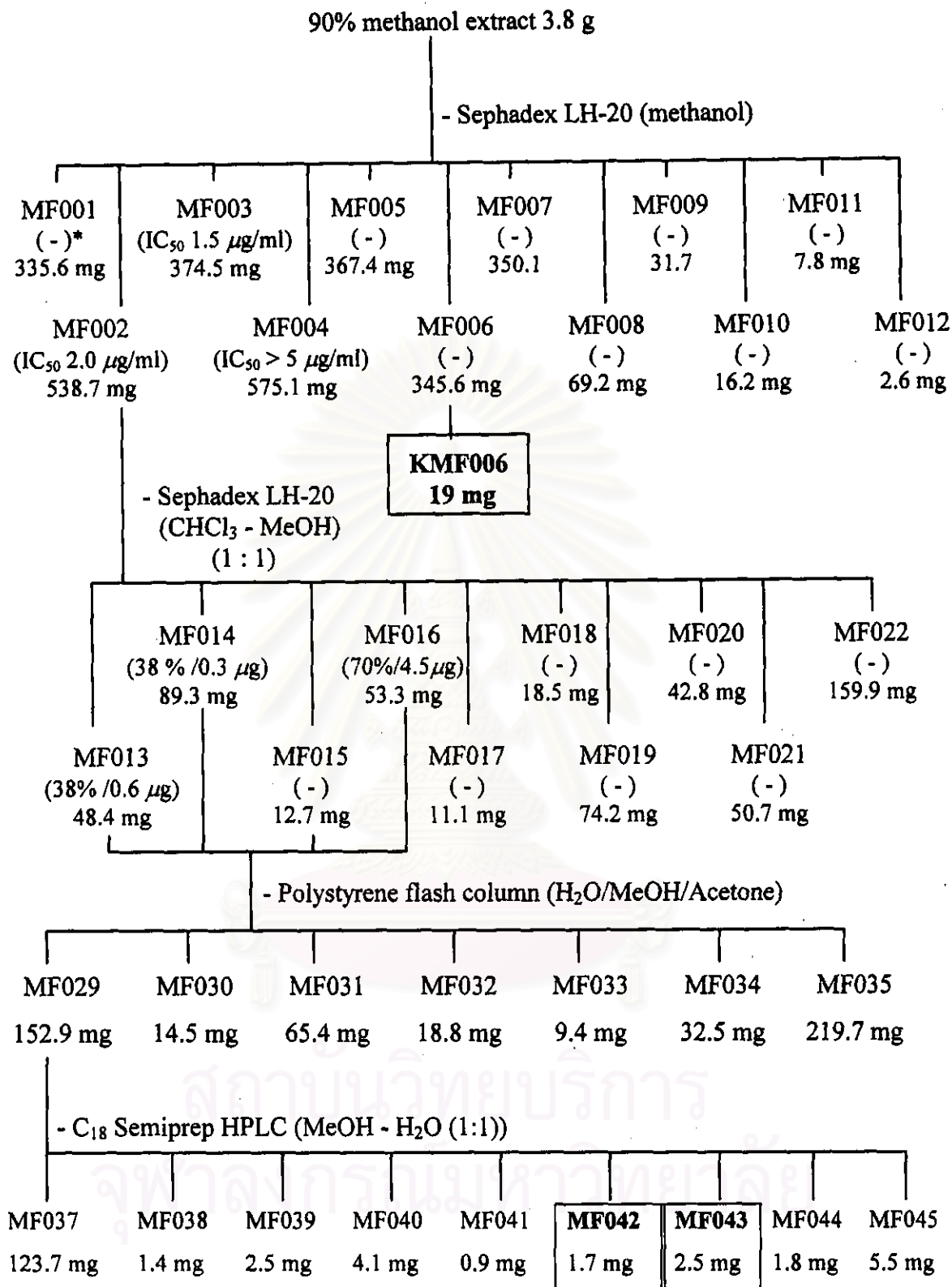
CHAPTER IV

RESULTS AND DISCUSSION

Isolation of the chemical constituents

The white crystal KMF006 (19 mg, 9.0×10^{-4} % of wet weight) was obtained from fraction MF006 by crystallization using CHCl_3 - methanol (1:1) (Scheme 3). The data from nmr and mass spectrum of fraction KMF006 indicated that this fraction is a mixture of two compounds. The major compound showed $[\text{M}+\text{H}]^+$ peak at m/z at 377 and was codenamed as KMF006-1, while the minor compound, which showed isotope peaks at m/z 329 and 331, was codenamed as KMF006-2. Both compounds produced yellow color with anisaldehyde-sulphuric acid and showed quenching spot under uv light at λ 254 nm with R_f value of 0.6 (Figure 11).

The compounds MF042 (1.7 mg, 8.0×10^{-5} % of wet weight) and MF043 (2.5 mg, 1.1×10^{-4} % of wet weight) were obtained from fraction MF002 by using gel filtration chromatography, polystyrene flash column chromatography and reversed phase semipreparative HPLC, respectively. Both compounds were brown powder.



* the bioactivity is in parenthesis

Scheme 3. Isolation of the compounds KMF006-1, KMF006-2, MF043 and MF042

Identification and structure elucidation of the isolated compounds

1. Compound KMF006-1

The mass spectrum of KMF006-1 (Figures 23-24) showed the protonated molecular ion peak at m/z 377, a fragment ion at m/z 261 due to the loss of one molecule of deoxy sugar, a fragment ion at m/z 134 due to further loss of iodide. The mass spectrum also showed a fragment ion at m/z 117 due to the loss of purine base. The ^1H nmr spectrum of KMF006-1 (Figures 12-14) showed 13 proton signals including two olefinic protons, four methine protons, two hydroxy groups and one methyl group. The ^{13}C nmr spectrum of KMF006-1 (Figure 15) showed 11 carbon signals including six sp^2 carbons, four oxygenated sp^3 carbons and one sp^3 carbon. These suggested KMF006-1 is a nucleoside of an iododeazapurine base with the molecular formula of $\text{C}_{11}\text{H}_{13}\text{IN}_4\text{O}_3$.

The complete carbon assignments of compound KMF006-1 were achieved by the analysis of the ^1H -detected Heteronuclear Multiple Quantum Coherence (HMQC) and ^1H -detected Heteronuclear Multiple Bond Quantum Coherence (HMBC) spectra, which provide the correlations between protons and carbons through one bond and long range couplings, respectively.

According to the HMQC spectrum (Figure 16), the protonated carbons could be assigned as follows: H-2 (δ 8.10 ppm) - C-2 (δ 152.0 ppm), H-6 (δ 7.59 ppm) - C-6 (δ 126.8 ppm), H-1' (δ 5.99 ppm) - C-1' (δ 86.9 ppm), H-2' (δ 4.38 ppm) - C-2' (δ 73.3 ppm), H-3' (δ 3.83 ppm) - C-3' (δ 74.5 ppm), H-4' (δ 3.88 ppm) - C-4' (δ 79.1 ppm) and 3H-5' (δ 1.26 ppm) - C-5' (δ 19.0 ppm).

The quaternary carbon assignments were determined by the long range coupling observed from the HMBC spectra (Figures 17-20). The signal at δ 157.1 ppm was assigned as C-4 by the long range coupling with H-2 (δ 8.10 ppm). The signal at δ 103.1 ppm was assigned as C-4a by the long range coupling with H-6 (δ 7.59 ppm). The signal at δ 52.2 ppm was assigned as C-5 by the two-bond coupling with H-6 (δ 7.59 ppm). The upfield shift of C-5 is due to the heavy atom iodine. The signal at δ 150.3 ppm was assigned as C-7a by the long range coupling with H-2 (δ 8.10 ppm), H-6 (δ 7.59 ppm) and H-1' (δ 5.99 ppm).

The relative configuration of the sugar part was proven by Nuclear Overhauser Effect (NOE) difference spectrum (Table 11). The NOE shows correlation among protons through space, by increasing the intensity of proton signals which are correlated to the irradiated protons.

Methyl group, H-2' and H-3' were proven to be the same β -oriented as the nucleoside base by the following results: the 2.5 % NOE at H-2, 3.1 % NOE at H-6 and 3.9 % NOE at H-2' upon irradiation of 5'-CH₃ (δ 1.25 ppm) (Figure 21), the 11.4 % NOE at H-6, 6.9 % NOE at H-3' and NOE at 5'-CH₃ upon irradiation of H-2' (δ 4.37 ppm) (Figure 21), the NOE at H-6, the NOE at H-2' and the NOE at 5'-CH₃ upon irradiation of H-3' (δ 3.82 ppm) (Figure 22), whereas no NOE was observed at H-4' and H-1' upon irradiation of H-2' and 5'-CH₃, respectively.

H-1' and H-4' are α -oriented and situated on the same face of the ring based on the following evidence: the 3.0 % NOE at H-4' upon irradiation of H-1' (δ 5.99 ppm) (Figure 21).

The close proximity of H-1' and H-2' through the quasiaxial and quasiequatorial orientations of the tetrahydrofuran ring were observed through the 4.3 % NOE at H-2' upon irradiation of H-1'.

Table 10. Carbon and proton chemical shift assignment and carbon-proton long range correlations of compound KMF006-1.

	δC (ppm)	δH (ppm)	long-range correlations in HMBC spectrum
1	-	-	-
2	152.0	8.10 (1H, s)	C-4, C-7a
3	-	-	-
4	157.1	-	-
4a	103.1	-	-
5	52.2	-	-
6	126.8	7.59 (1H, s)	C-4a, C-5, C-7a, C-1'
7	-	-	-
7a	150.3	-	-
4-NH ₂	-	6.64 (2H, br s)	-
1'	86.9	5.99 (1H, d, $J = 5.5$ Hz)	C-6, C-7a, C-2'
2'	73.3	4.38 (1H, q, $J = 5.5$ Hz)	C-1', C-4'
3'	74.5	3.83 (1H, q, $J = 5.5$ Hz)	C-1', C-5'
4'	79.1	3.88 (1H, p, $J = 5.5$ Hz)	C-2', C-3'
5'	19.0	1.26 (3H, d, $J = 6.4$ Hz)	C-3', C-4'
2'-OH	-	5.29 (1H, d, $J = 5.5$ Hz)	C-1', C-2'
3'-OH	-	5.07 (1H, d, $J = 5.5$ Hz)	C-2', C-3, C-4'

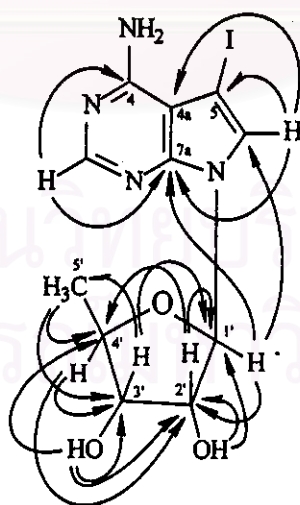


Figure 5. The long-range correlations in the HMBC spectrum of compound KMF006-1

The 1.3 % and 2.5 % enhancement of the intensity of H-2 signal upon irradiation of H-1' and 5'-CH₃, respectively, and the 3.0, 11.4 and 3.1 % enhancement of the intensity of H-6 signal upon irradiation of H-1', H-2' and 5'-CH₃, respectively, suggested that two orientations (I, II) of the purine base exist as shown. H-2 is facing outward of the sugar part in form I while facing toward the sugar part in form II. The rotation of C-1'-N-7 bond makes these two form quickly interconvertible.

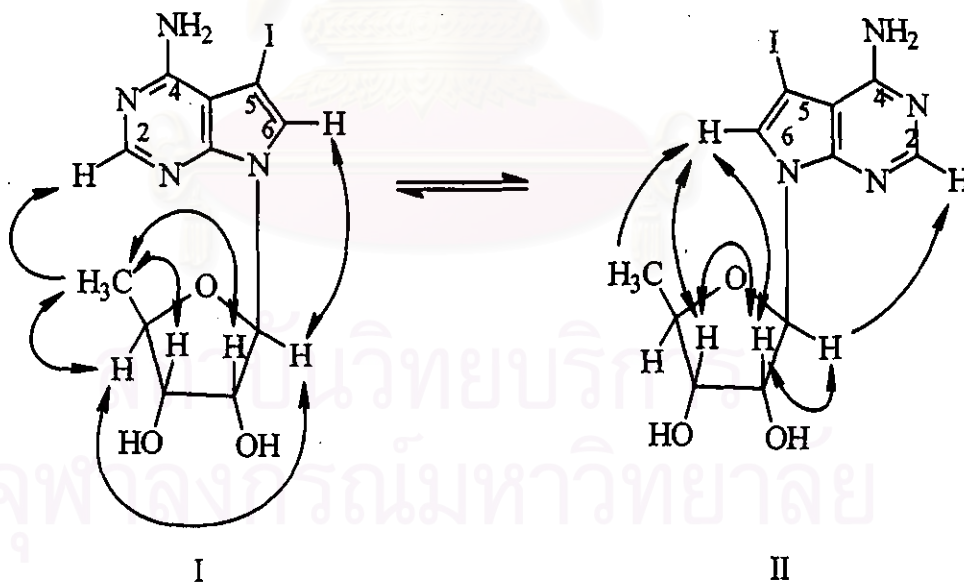
Observation of the coupling of the protons within the sugar moiety revealed correlation between H-1' doublet at δ 5.99 ppm and H-2' with the coupling constant of 5.5 Hz. The H-2' quartet at δ 4.38 ppm showed coupling with H-1', H-3' and 2'-OH with the coupling constant of 5.5 Hz. The H-3' quartet at δ 3.83 ppm, by turns, showed coupling with H-4' and 3'-OH with the same coupling constant. The H-4' pentet at δ 3.88 ppm further coupled with H-5' doublet at δ 1.26 ppm with the coupling constant of 6.4 Hz.

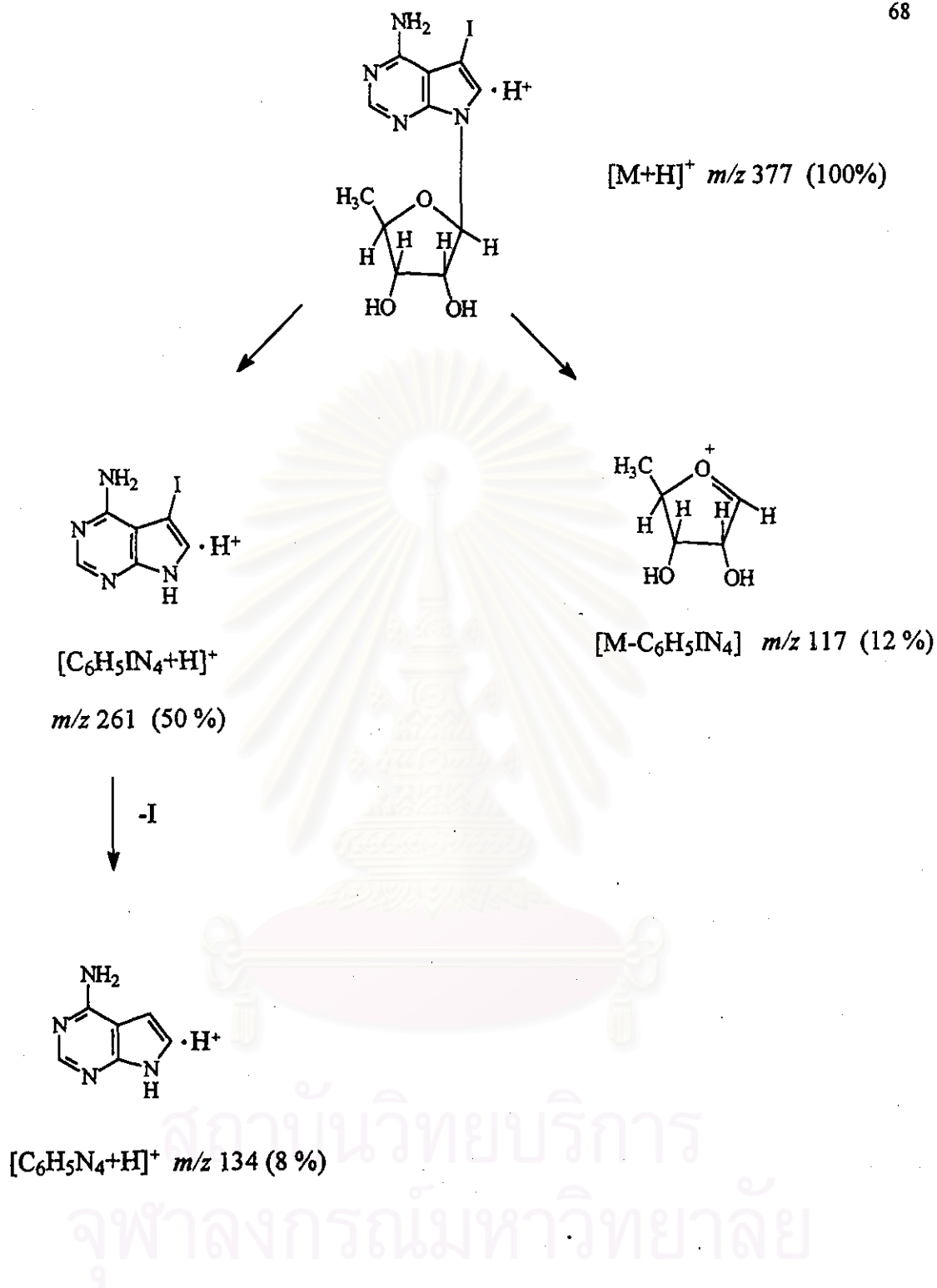
Therefore, the sugar part was recognized as 5-deoxyribose β -linked to the base. This was also supported by comparison of its ¹³C nmr spectrum with the literature (Kazlauskas *et al.*, 1983).

By comparison of spectral data, the compound KMF006-1 was shown to be identical to the previously reported compound, 4-amino-7-(5'-deoxyribos-1' β -yl)-5-iodopyrrolo[2,3-*d*]pyrimidine [42], isolated from a red alga, *Hypnea valendiae* (Kazlauskas *et al.*, 1983). This compound produced potent muscle relaxation and hypothermia in mice and extremely potent inhibitor of adenosine kinase from guinea-pig brain and rat brain and liver (Davies *et al.*, 1984).

Table 11. NOE enhancements for compound KMF006-1.

	% NOE						
	H-2	H-6	H-1'	H-2'	H-3'	H-4'	H-5'
H-6	-		+ (3.2)	+ (6.4)	+ (1.0)	-	-
H-1'	+ (1.3)	+ (3.0)		+ (4.3)	-	+ (3.0)	-
H-2'	-	+ (11.4)	+ (4.8)		+ (6.9)	-	+
H-3'	-	+	-	+		-	+
H-4'	-	-	+	-	-		+
H-5'	+ (2.5)	+ (3.1)	-	+ (3.9)	+ (6.3)	+ (18.7)	

**Figure 6.** Correlations in the NOE difference spectra of compound KMF006-1



Scheme 4. Proposed mass fragmentation of compound KMF006-1

2. Compound KMF006-2

The mass spectrum of KMF006-2 (Figures 25-26) showed the protonated isotope molecular ion peaks at m/z 329 and 331, suggesting the presence of a single bromine atom, a fragment ions at m/z 215 and 213 due to the loss of one molecule of deoxy sugar, a fragment ion at m/z 134 due to further loss of a bromine atom. The mass spectrum also showed a fragment ion at m/z 117 due to the loss of purine base.

The ^1H nmr spectrum of KMF006-2 (Figures 12-14) showed 13 proton signals including two olefinic protons, four methine protons, two hydroxy groups and one methyl group. The ^{13}C nmr spectrum of KMF006-2 (Figure 15) showed six sp^2 carbons, four oxygenated sp^3 carbons and one sp^3 carbon.

The 5'-deoxypentofuranose sugar part was observed through coupling of H-1' doublet at δ 6.01 ppm to H-2' quartet at δ 4.37 ppm with the coupling constant of 5.5 Hz, which, by turns, coupling with H-3' and 2'-OH with similar coupling constant. The H-3' quartet at δ 3.83 ppm further showed coupling with H-4' and 3'-OH with the same coupling constant of 5.5 Hz. Finally, the H-4' pentet at δ 3.89 ppm also showed coupling with H-5' with the coupling constants of 6.4 Hz.

The complete carbon assignments of compound KMF006-2 were achieved by the analysis of the HMQC and HMBC spectra. According to HMQC spectrum (Figure 16), the protonated carbons could be assigned as follows; H-2 (δ 8.10 ppm) - C-2 (δ 152.5 ppm), H-6 (δ 7.59 ppm) - C-6 (δ 121.6 ppm), H-1' (δ 6.01 ppm) - C-1' (δ 87.0 ppm), H-2' (δ 4.37 ppm) - C-2' (δ 73.3 ppm), H-3' (δ 3.83 ppm) - C-3' (δ 74.5 ppm), H-4' (δ 3.89 ppm) - C-4' (δ 79.2 ppm) and 3H-5' (δ 1.26 ppm) - C-5' (δ 19.0 ppm).

Table 12. Carbon and proton chemical shift assignments and carbon-proton long range correlations of Compound KMF006-2.

	δC (ppm)	δH (ppm)	long-range correlations in HMBC spectrum
1	-	-	-
2	152.5	8.10 (1H, s)	C-4, C-7a
3	-	-	-
4	156.9	-	-
4a	100.9	-	-
5	87.1	-	-
6	121.6	7.58 (1H, s)	-
7	-	-	-
7a	149.8	-	-
4-NH ₂	-	6.64 (2H, br s)	-
<hr/>			
1'	87.0	6.01 (1H, d, $J = 5.5$ Hz)	C-6, C-7a, C-2'
2'	73.3	4.37 (1H, q, $J = 5.5$ Hz)	-
3'	74.5	3.83 (1H, q, $J = 5.5$ Hz)	-
4'	79.2	3.89 (1H, p, $J = 5.5$ Hz)	-
5'	19.0	1.26 (3H, d, $J = 6.4$ Hz)	-
2'-OH	-	5.31 (1H, d, $J = 5.5$ Hz)	-
3'-OH	-	5.08 (1H, d, $J = 5.5$ Hz)	-

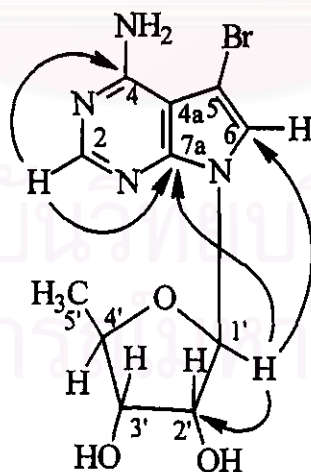
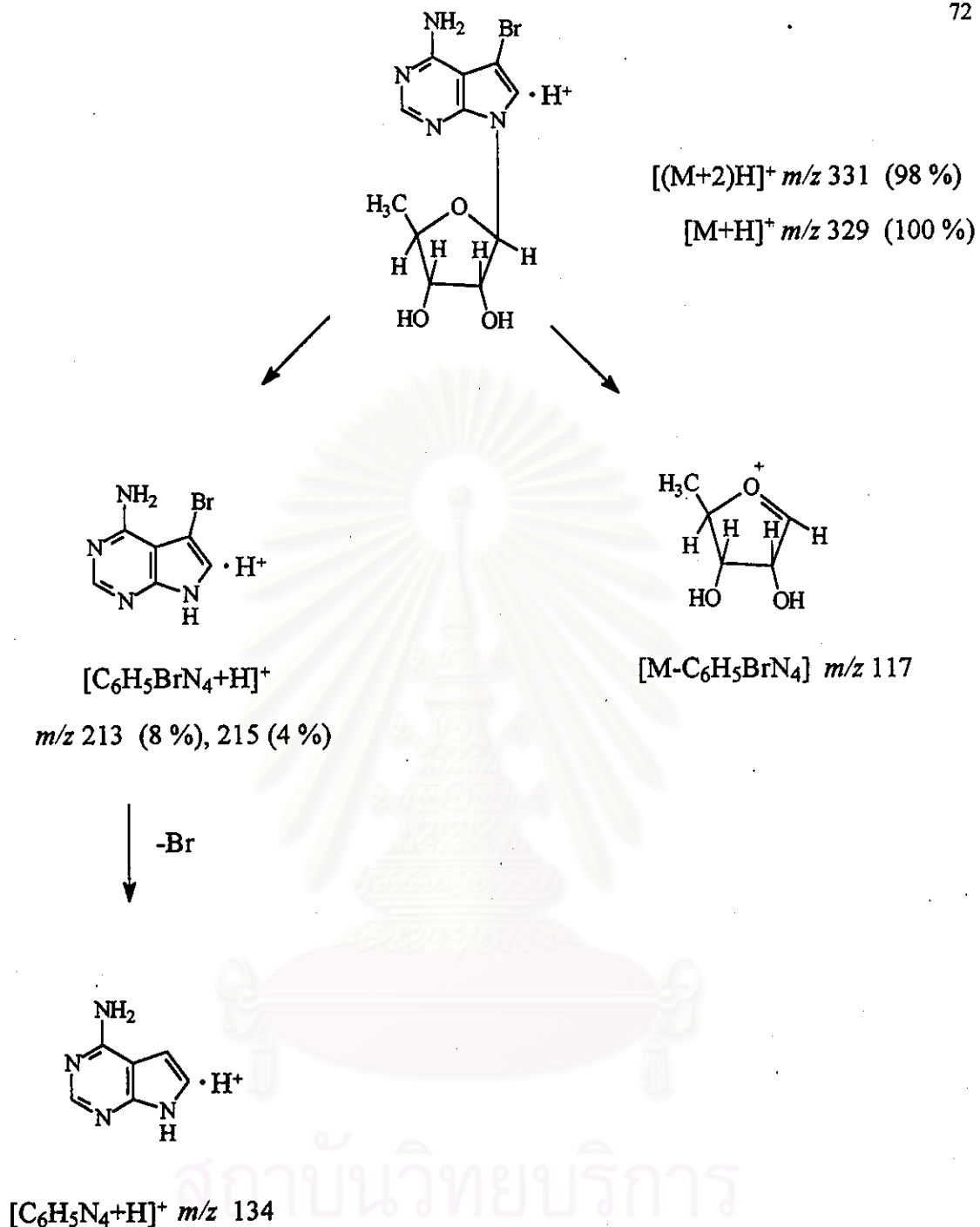


Figure 7. The long-range correlations in the HMBC spectrum of compound KMF006-2

The quaternary carbons assignments were determined by the long range coupling observed from the HMBC spectra (Figures 17-20). The signal at δ 156.9 ppm was assigned as C-4 by the long range coupling with H-2 (δ 8.10 ppm). The signal at δ 149.8 ppm was assigned as C-7a by the long range coupling with H-2 (δ 8.10 ppm) and H-1' (δ 6.01 ppm).

By comparison of spectral data with compound KMF006-1, the sugar part of compound KMF006-2 was shown to be the same as compound KMF006-1. There is a bromine atom substituted at C-5 of aminodeazapurine base. The structure of compound KMF006-2 should therefore be 4-amino-7-(5'-deoxyribos-1' β -yl)-5-bromopyrrolo[2,3-*d*]pyrimidine, which is identical to a known compound [41] previously isolated from the ascidian *Didemnum voeltzkowi* (Mitchell *et al.*, 1996). Its molecular formula should be $C_{11}H_{13}BrN_4O_3$.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย



Scheme 5. Proposed mass fragmentation of compound KMF006-2

3. Compound MF043

The mass spectrum of MF043 (Figures 39-40) showed the protonated molecular ion peak at m/z 377, a fragment ion at m/z 261 due to the loss of one molecule of deoxy sugar, a fragment ion at m/z 134 due to further loss of iodide. The mass spectrum also showed a fragment ion at m/z 117 due to the loss of purine base.

The ^1H nmr spectrum of MF043 (Figures 30-31) showed 13 proton signals including two olefinic protons, four methine protons and one methyl group. The ^{13}C nmr spectrum of MF043 (Figure 32) showed 11 carbon signals including six sp^2 carbons, four oxygenated sp^3 carbons and one sp^3 carbon.

The 5'-deoxypentofuranose sugar part could be observed through coupling of H-1' doublet at δ 5.95 ppm to H-2' broad singlet with the coupling constant of 1.5 Hz. The H-2' also showed coupling with H-3' with a small coupling constant. The H-3' doublet at δ 3.81 ppm further coupled to H-4' with the coupling constant of 3.4 Hz. The H-4' doublet of quartets at δ 4.20 ppm also showed coupling with and H-5' with the coupling constant of 6.4 Hz.

The complete carbon assignments of compound MF043 were achieved by the analysis of the HSQC and HMBC spectra. According to HSQC spectrum (Figure 33), the protonated carbons were assigned as follows: H-2 (δ 8.10 ppm) - C-2 (δ 151.9 ppm), H-6 (δ 7.60 ppm) - C-6 (δ 128.0 ppm), H-1' (δ 5.95 ppm) - C-1' (δ 89.3 ppm), H-2' (δ 4.13 ppm) - C-2' (δ 81.9 ppm), H-3' (δ 3.81 ppm) - C-3' (δ 76.5 ppm), H-4' (δ 4.20 ppm) - C-4' (δ 77.9 ppm) and 3H-5' (δ 1.20 ppm) - C-5' (δ 14.0 ppm).

Table 13. Carbon and proton chemical shift assignments and carbon-proton long range correlations of compound MF043.

	δC (ppm)	δH (ppm)	long-range correlations in HMBC spectrum
1	-	-	-
2	151.9	8.10 (1H, s)	C-4, C-7a
3	-	-	-
4	157.2	-	-
4a	102.8	-	-
5	51.3	-	-
6	128.0	7.60 (1H, s)	C-4a, C-5, C-7a
7	-	-	-
7a	149.5	-	-
4-NH ₂	-	6.65 (2H, br s)	-
<hr/>			
1'	89.3	5.95 (1H, d, $J = 1.5$ Hz)	C-6, C-7a, C-2'
2'	81.9	4.13 (1H, br s)	C-4'
3'	76.5	3.81 (1H, br d, $J = 3.4$ Hz)	-
4'	77.9	4.20 (1H, dq, $J = 6.4, 3.4$ Hz)	-
5'	14.0	1.20 (3H, d, $J = 6.4$ Hz)	C-3', C-4'

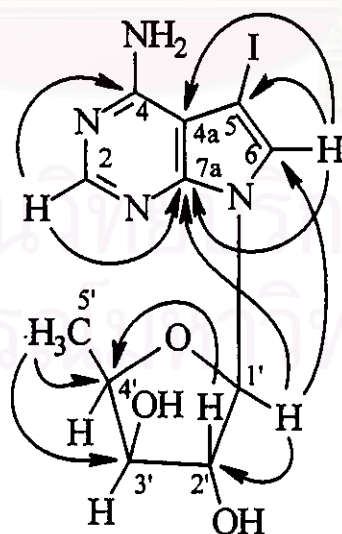


Figure 8. The long-range correlations in the HMBC spectrum of compound MF043

The quaternary carbons assignments were supported by the long range coupling observed from the HMBC spectra (Figure 34). The signal at δ 157.2 ppm was assigned as C-4 by its long range coupling with H-2 (δ 8.10 ppm). The signal at δ 102.8 ppm was assigned as C-4a by the long range coupling with H-6 (δ 7.60 ppm). The signal at δ 51.3 ppm was assigned as C-5 by the long range coupling with H-6 (δ 7.60 ppm). The signal at δ 149.5 ppm was assigned as C-7a by the long range coupling with H-2, H-6 and H-1' at δ 8.10, 7.60 and 5.95 ppm, respectively.

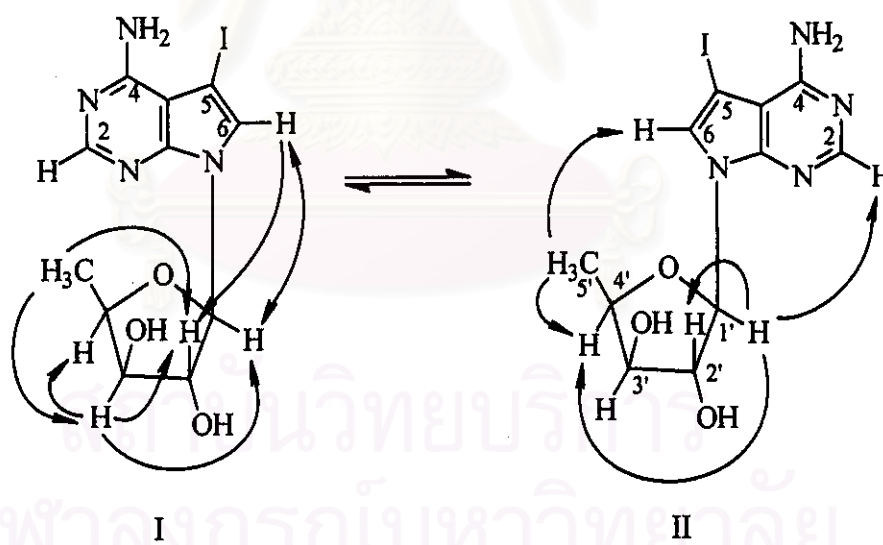
The relative configuration of the sugar part is demonstrated by Nuclear Overhauser Effect (NOE) difference spectra (Table 14). Methyl group and H-2' were proven to be the same β -oriented as the nucleoside base by the following observations: the NOE at H-6 and H-2' upon irradiation of 5'-CH₃ at δ 1.21 ppm (Figure 38) and the absence of NOE at H-3' and H-1' upon irradiation of H-6 at δ 7.60 ppm (Figure 35) and 5'-CH₃ at δ 1.21 ppm, respectively.

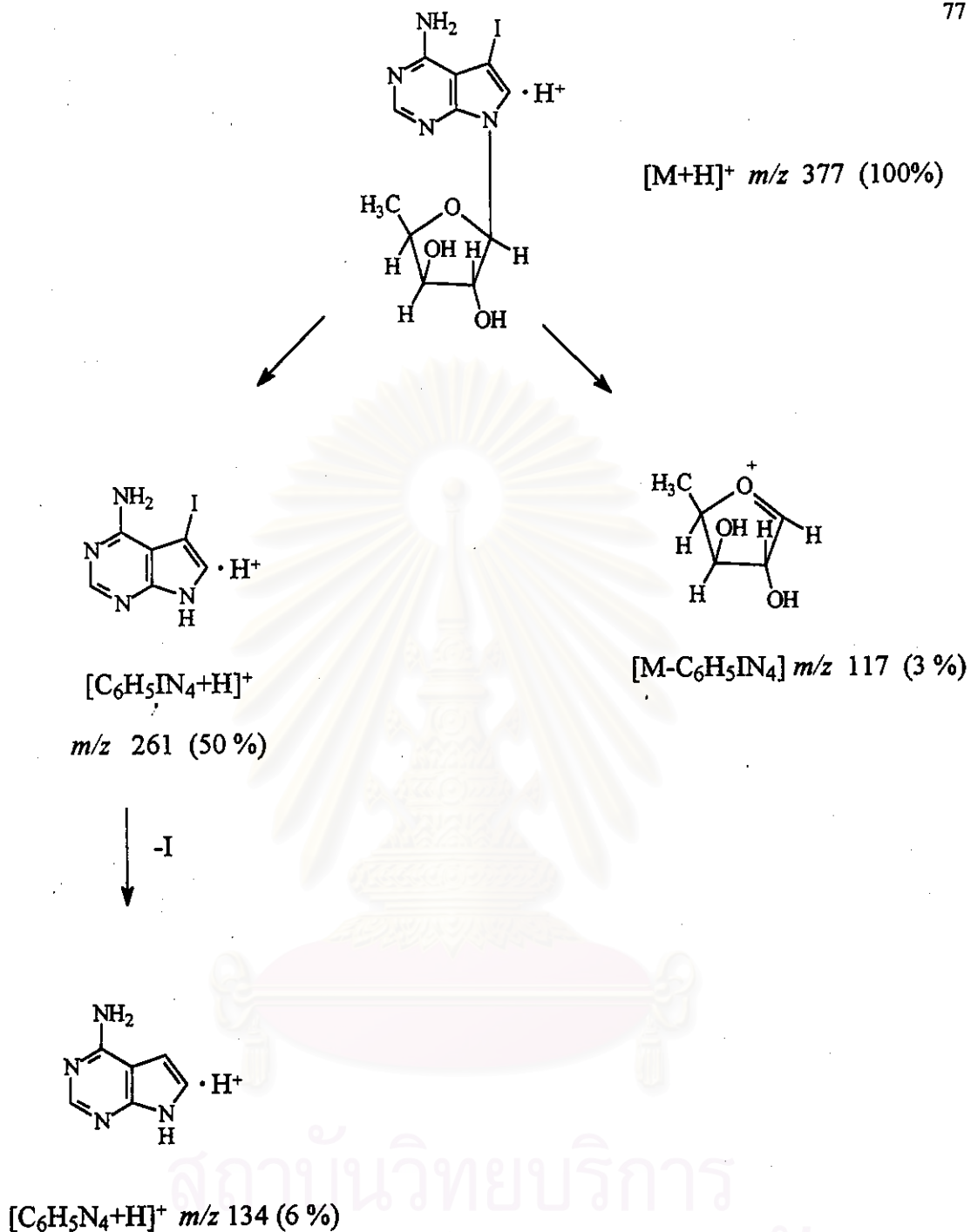
H-1', H-3' and H-4' are α -oriented and situated on the same face of the ring based on the following evidence: the 1.5 % NOE at H-4' upon irradiation of H-1' at δ 5.95 ppm (Figure 36) and the 1.0 % NOE at H-1' upon irradiation of H-3' at δ 3.82 ppm (Figure 37). This indicated that the sugar part is 5'-deoxyxylofuranose.

The spectral data of compound MF043 was found to be identical to the previously isolated compound identified as 4-amino-7-(5'-deoxyribos-1' α -yl)-5-iodopyrrolo[2,3-*d*]pyrimidine [43] from the red alga *Hypnea valendiae* (Kazlauskas *et al.*, 1983). However, NOE spectral data indicated that the structure of this compound should be revised to 4-amino-7-(5'-deoxyxylos-1' β -yl)-5-iodopyrrolo[2,3-*d*]pyrimidine. Its molecular formula should be C₁₁H₁₃IN₄O₃.

Table 14. NOE enhancements for compound MF043.

	% NOE						
	H-2	H-6	H-1'	H-2'	H-3'	H-4'	H-5'
H-6	-		+ (3.4)	+ (4.7)	-	-	-
H-1'	+	+ (2.7)		+ (2.1)	-	+ (1.5)	-
H-3'	-	-	+ (1.0)	+ (1.8)		+ (6.8)	+
H-5'	-	+	-	+	+	+	

**Figure 9.** Correlations in the NOE difference spectrum of compound MF043



Scheme 6. Proposed mass fragmentation of compound MF043

4. Compound MF042

The mass spectrum of MF042 (Figures 44-45) displayed the protonated isotope molecular ion peak at m/z 329 and 331, suggesting the presence of a single bromine atom. Fragment ions at m/z 215 and 213 were due to the loss of one molecule of deoxy sugar, while another at m/z 134 indicated further loss of a bromine atom. The mass spectrum also showed the loss of purine base, producing a fragment ion at m/z 117.

The ^1H nmr spectrum of MF042 (Figures 42-43) was similar to that of MF043, showing 13 proton signals including two olefinic protons, four methine protons and one methyl group.

The 5'-deoxypentofuranose sugar part could be observed through coupling of H-1' doublet at δ 5.98 ppm to H-2' singlet at δ 4.13 ppm with the coupling constant of 1.5 Hz. H-2' alternately coupled with H-3' with a small coupling constant. The H-3' doublet at δ 3.82 ppm could also be observed as coupled to H-4' with a coupling constant of 3.4 Hz. The H-4' doublet of quartets at δ 4.21 ppm showed coupling with H-3' and H-5' with the coupling constants of 3.4 and 6.4 Hz, respectively.

Comparison of the chemical shift, coupling constant and mass spectral data of compound KMF006-2 with MF043 suggested the structure of the latter as the novel 4-amino-7-(5'-deoxyxylos-1' β -yl)-5-bromopyrrolo[2,3-*d*]pyrimidine (Figure 10). Its molecular formula should therefore be $\text{C}_{11}\text{H}_{13}\text{BrN}_4\text{O}_3$.

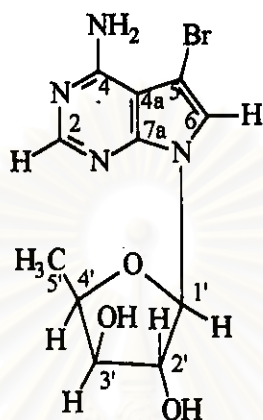
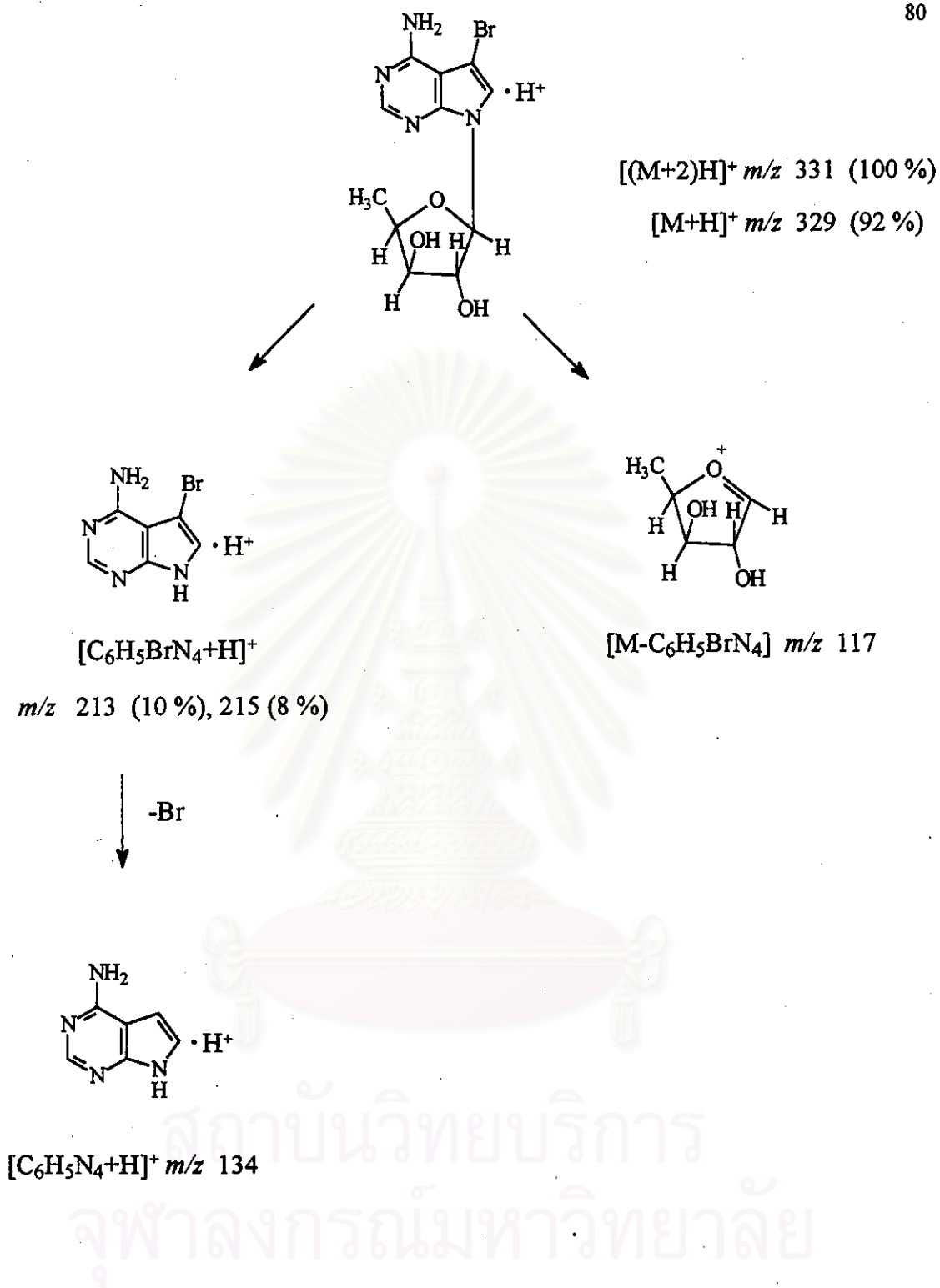


Figure 10. Structure of MF042

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย



Scheme 7. Proposed mass fragmentation of compound MF042