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Synthesis, isolation, and characterization of ABT-578 equilibrium isomers

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Abstract—ABT-578, an anti-restenosis agent exists as two isomers, a major pyran form and a minor oxepane form. The existence of the two isomeric forms was established by isolation and equilibration studies under buffered and physiological conditions. Finally their structures were confirmed by converting the major pyran form to the oxepane form by synthesis, isolation, and characterization.

ABT-578 [40-epi-(1-tetrazolyl)-rapamycin] is a tetrazole containing semi-synthetic rapamycin analog that has been developed to coat cardiovascular device stents (drug eluting stent, DES) to minimize restenosis in angioplasty patients.[1] ABT-578 is a potent inhibitor of T-cell lymphocyte proliferation similar to rapamycin.[2,3]

The rapamycin analog, ABT-578 exists in two isomeric forms, a major pyran (6-member isomer at 10-position) and a minor oxepane isomer (7-member isomer at 9-position), that are in equilibrium with each other in a ratio of ~10:1 as shown in Figure 1.

Reverse phase analysis of ABT-578 on a C-18 or phenyl column indicated that the major isomer, which eluted earlier, is the 6-membered pyran form **1**. The minor component, the oxepane isomer **2** eluted 3–4 min later (Fig. 2). On a normal phase HPLC (YMC silica gel, 5 μm, 250 × 4.6 mm), the two forms did not have baseline resolution, however under optimal conditions the oxepane form eluted prior to the pyran form.

In order to demonstrate the equilibrium between the two isomers, each form was isolated by multiple HPLC injections of ABT-578 on a reverse phase phenyl column under pH 4 buffered conditions. Utilizing this protocol, the pyran and oxepane isomers could each be obtained in solution in an initial 99:1 isomeric ratio. The equilibrium nature of the two forms was then clearly established by monitoring their interconversion over time by HPLC analysis. As shown in Table 1, at pH 4 the pyran form reached equilibrium of 90:9 over 120 h, while the oxepane form was equilibrated to 83:16. There was some formation of open ring acid **3** during the study. These results clearly indicate that the two forms are under equilibrium, with the pyran form (**1**) being more thermodynamically stable.

In another set of experiments, pure oxepane **2**, (prepared as described in Fig. 3) was dissolved in a pH 7.4 ammonium acetate buffer/acetonitrile mixture (physiological pH) and the equilibration monitored by HPLC at 1 h intervals (Table 2). Under these conditions the equilibration was rapid with the pyran form predominating after as little as 2 h and equilibrium reached in ~7 h. The effect of pH on the equilibration rate of the isomeric forms is consistent with that reported for rapamycin.[4]

In order to obtain oxepane isomer **2** in solid form for characterization it was imperative to isolate this isomer. Thus, a solution of ABT-578 was treated with excess of hindered Grignard reagent (*t*-butyl magnesium bromide in THF solution) at 0–25 °C. The reaction mixture was quenched with 1 N HCl, and then extracted with ethyl acetate. The organic phase was washed with water, dried (sodium sulfate), and concentrated. The HPLC of the

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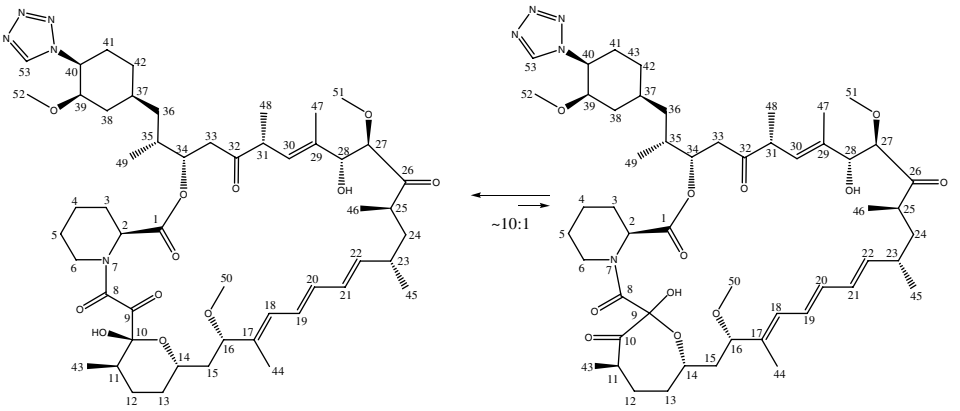

Pyran **1** Oxepane **2**

Figure 1. Isomeric Forms of ABT-578.

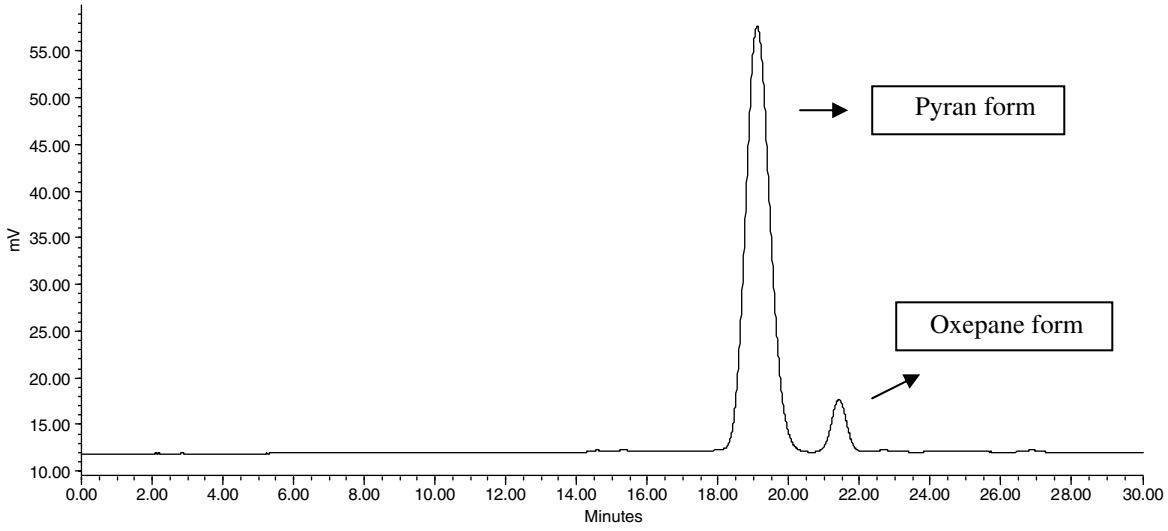

Figure 2. ABT-578 isomeric forms on a reverse phase phenyl column.

Table 1. Equilibration studies of the pyran **1** and the oxepane **2** forms at pH 4

Equilibration at pH 4			
Pyran **1**		Oxepane **2**	
After hours	Ratio **1/2**	After hours	Ratio **1/2**
1.5	99:1	0.5	1:99
3.5	98:2	3.5	18:82
5.5	97:3	5.5	27:71
7.5	96:4	7.5	36:63
50	92:8	50	70:28
120	90:9	120	83:16

Table 2. Equilibration studies with oxepane isomer **2** at pH 7.4

Hours	Pyran **1**	Oxepane **2**
0	3.9	96.1
1	42.7	57.2
2	63.7	36.3
5	81.6	18.4
6	82.6	17.3
7	83.1	16.9
10	83.6	16.4
72	83.9	16.1

crude reaction mixture showed the presence of >65% of the oxepane form, <6% of the pyran form, and a minor amount of the open ring acid **3** byproduct, a major metabolite of ABT-578.

When a less hindered base, benzyl magnesium bromide was used,[5] the formation of open ring acid **3** was observed in higher yields (>20%), most probably via a β-elimination process. The crude reaction mixture was purified on a C-18 semi-prep column. The oxepane and pyran forms were isolated in excess of 98% purity by HPLC. A proposed mechanism for the conversion of the pyran to oxepane[5] form with the assistance of a Grignard reagent is illustrated in Figure 3.

The structural elucidation of the two forms was performed using ^{1}H, ^{13}C, g-DQCOSY, g-HSQC, and g-HMBC experiments (500 MHz (^{1}H NMRs) and 400 MHz (^{13}C NMRs)). There are two amide bond rotamers

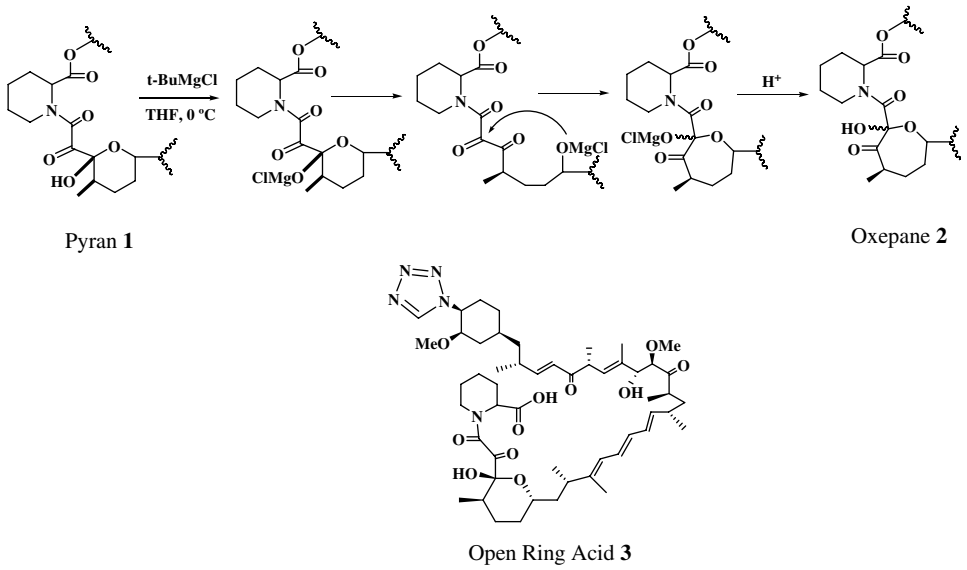

Figure 3. Grignard reagent mediated conversion of the pyran to the oxepane form.

Table 3. Partial ^{1}H and ^{13}C chemical shift assignments of the pyran and the oxepane forms

No.	Group	Pyran **1**		Oxepane **2**	
		^{1}H (ppm)[a,c]	^{13}C (ppm)[b,c]	^{1}H (ppm)[a,d]	^{13}C (ppm)[b,d]
1	C=O	—	169.13 (168.74)	—	169.93 (170.43)
2	CH	4.94 (4.25)	50.62 (56.04)	4.97 (5.57)	51.18 (55.01)
6	CH_2	3.20, 3.43 (2.86, 4.27)	43.44 (38.12)	4.56, 2.72 (4.14, 2.90)	42.74 (38.09)
8	C=O	—	167.05 (166.17)	—	167.90 (167.77)
9	C	—	199.05 (198.52)	—	98.07 (98.58)
10/9	OH	6.44	—	7.30 (7.22)	
10	C	—	99.02 (98.68)	—	209.85 (209.38)
11	CH	2.03	34.81	3.05	42.64
14	CH	4.00 (3.74)	66.24 (66.45)	3.71 (3.36)	71.74 (73.17)

[a] The residual signal of DMSO-d_6 assigned to 2.50 ppm.
[b] The residual signal of DMSO-d_6 assigned to 39.5 ppm.
[c] The values in parentheses are assignments for the cis rotamers, where these can be distinguished.
[d] Both amide bond rotamers are listed. The trans and cis rotamers may be switched due to the similar concentrations.

in solution for both pyran and oxepane forms of ABT-578, whereas rapamycin occurs as ~3:1 amide bond rotamers. The trans rotamer of ABT-578 is predominant (~90%) for the pyran form,[6–8] whereas there are almost equal populations of the trans and cis rotamers for the oxepane form in DMSO-d_6. All of the correlations in 2D spectra (g-DQCOSY, g-HSQC, and g-HMBC) are consistent with the structural assignments for the pyran and the oxepane forms. The partial chemical shifts of the pipecolinyl ring, dicarbonyl, and hemi-ketal ring regions are listed in Table 3. The complete ^{1}H and ^{13}C NMR chemical shifts of ABT-578[9] are cited in Ref. 10.

The carbon chemical shifts of C-9, C-10, and C-14, as well as H-11 chemical shift in oxepane form are consistent with the oxepane of rapamycin.[5] The stereochemistry of the hydroxyl group at position 9 could not be determined as well as the trans and cis rotamers of oxepane form by NMR. The two forms (pyran and oxepane) showed a similar $(M-H)^-$ of 964.6 units.

In conclusion, the existence of ABT-578 in two isomeric forms has been discussed and established. The major pyran form **1** can be converted to the minor oxepane form **2** without significant formation of the open ring acid **3** product via elimination. The two forms were isolated by preparative HPLC purification, and their structures confirmed by ^{1}H and ^{13}C correlation NMR experiments. The pure pyran and the oxepane forms clearly interconvert at both pH 4 and physiological pH 7.4. Thus ABT-57 exists as two equilibrating isomeric forms, a 6-membered pyran **1** and a 7-membered oxepane **2**.

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10. The NMR spectrum of ABT-578 in DMSO-d_6 is described below:
    ^{1}H NMR (DMSO-d_6, position in bracket): ppm 0.73 (CH_3, 43); 0.81 (CH_3, 49); 0.84 (CH_3, 46); 0.89 (CH_3, 48); 0.98 (CH_3, 45); 1.41, 1.05 (H-24a, H-24b); 1.18, 1.10 (H-36a, H-36b); 1.52 (CH, 37); 1.53 (CH_2, 12 and 42); 1.59, 1.30 (H-5a, H-5b); 1.41, 1.67 (H-4a, H-4b); 1.11, 1.73 (H-38a, H-38b); 1.30, 1.86 (H-15a, H-15b); 1.21, 1.83 (H-13a, H-13b); 1.62 (CH_3, 44); 1.73 (CH_3, 47); 1.76 (CH, 35); 1.60, 2.09 (H-3a, H-3b); 1.93, 2.21 (H-41a, H-41b); 2.05 (CH, 11); 2.22 (CH, 23); 2.47 (CH, 25); 2.40, 2.77 (H-33a, H-33b); 3.06 (OCH_3, 50); 3.16 (OCH_3, 51); 3.22, 3.44 (H-6a, H-6b); 3.29 (OCH_3, 52); 3.29 (CH, 31); 3.60 (CH, 39); 3.62 (CH, 16); 3.89 (CH, 27); 4.01 (CH, 14); 4.02 (CH, 28); 4.95 (CH, 2); 5.02 (CH, 34); 5.10 (=CH, 30); 5.17 (CH, 40); 5.24 (OH, 28); 5.46 (=CH, 22); 6.09 (=CH, 18); 6.15 (=CH, 21); 6.21 (=CH, 20); 6.42 (=CH, 19); 6.42 (OH, 10), 9.30 (CH, 53).
    ^{13}C NMR (DMSO-d_6, position in bracket): ppm 10.4 (CH_3, 44); 13.1 (CH_3, 47); 13.6 (CH_3, 46); 14.5 (CH_3, 49); 15.5 (CH_3, 43 and 48); 20.3 (CH_2, 4); 21.6 (CH_3, 45); 24.4 (CH_2, 5); 26.2 (CH_2, 12); 26.4 (CH_2, 3); 26.8 (CH_2, 41); 27.2 (CH_2, 42); 29.6 (CH_2, 13); 31.6 (CH_2, 38), 31.7 (CH, 37); 32.9 (CH, 35); 34.8 (CH, 11); 35.2 (CH, 23); 38.2 (CH_2, 36); 39.1 (CH, 25); 39.4 (CH_2, 33); 39.6 (CH_2, 24), 40.0 (CH_2, 15); 43.4 (CH_2, 6); 45.2 (CH, 31); 50.6 (CH, 2); 55.4 (OCH_3, 50); 55.8 (OCH_3, 52); 57.0 (OCH_3, 51); 55.9 (CH, 40); 66.2 (CH, 14); 73.4 (CH, 34); 75.6 (CH, 28); 77.4 (CH, 39); 82.3 (CH, 16); 85.7 (CH, 27); 99.0 (C-10); 125.3 (=CH, 30); 127.0 (=CH, 18 and 19); 130.4 (=CH, 21); 132.2 (=CH, 20); 137.2 (=C–CH_3, 29); 137.7 (=C–CH_3, 17); 139.2 (=CH, 22); 144.6 (CH, 53); 167.0 (C=O, 8); 169.1 (C=O, 1); 199.0 (C=O, 9); 207.5 (C=O, 32); 210.7 (C=O, 26).