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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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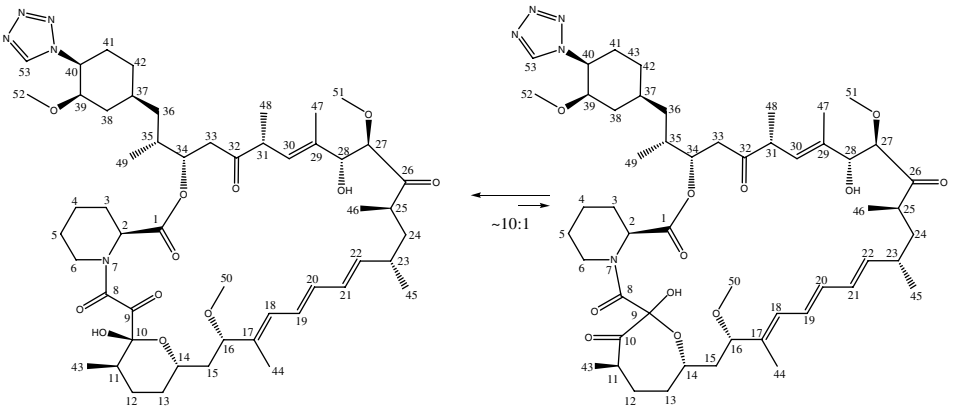


Pyran **1** Oxepane **2**

**Figure 1.** Isomeric Forms of ABT-578.

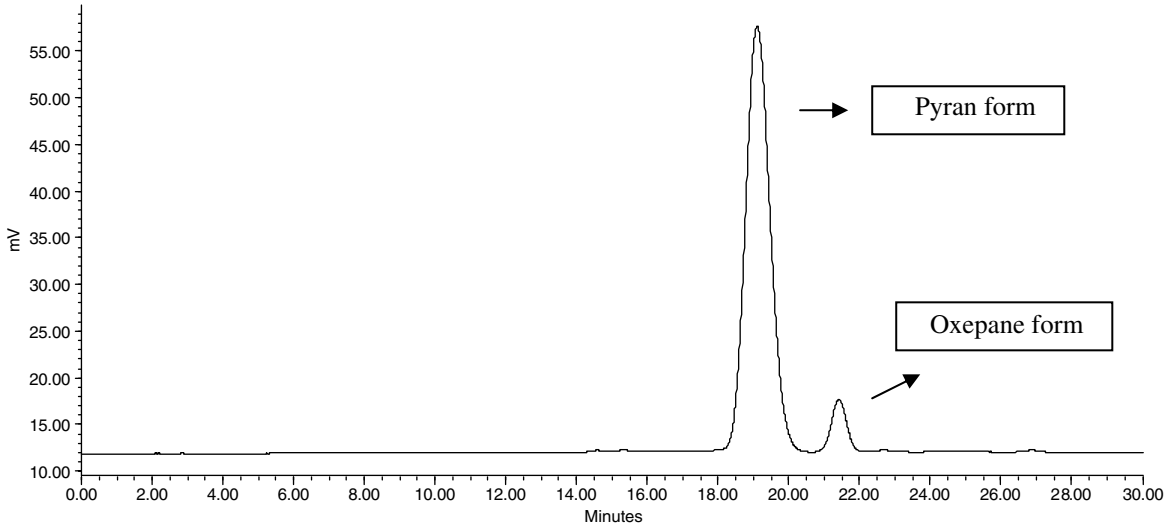


**Figure 2.** ABT-578 isomeric forms on a reverse phase phenyl column.

**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