JOC*Note*

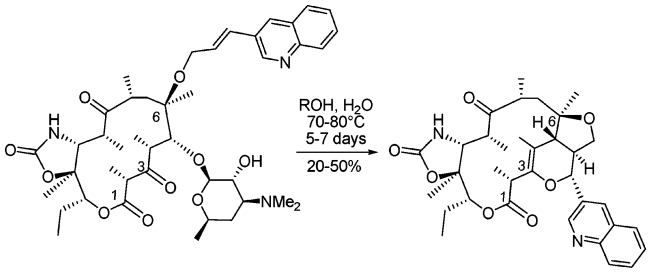
An Unusual Intramolecular Hetero-Diels–Alder Cycloaddition

Eric J. Stoner,* Michael S. Allen, Alan C. Christesen, Rodger F. Henry, L. Steven Hollis, Robert Keyes, Ian Marsden, Tamara C. Rehm, Sandeep G. Shiroor, Niru B. Soni, and Kent D. Stewart

Abbott Laboratories, 1401 Sheridan Road, North Chicago, Illinois 60064-6290

eric.stoner@abbott.com

Received January 3, 2005



A new reaction of erythronolides, an intramolecular hetero-Diels–Alder, has been discovered. Heated aqueous alcoholic solutions of ABT-773 (**1**) and its cis isomer (**3**) convert slowly to cycloadducts **2** and **4**, respectively. Optimal reaction conditions, mechanistic studies supported by molecular modeling, and biological activity data are reported. Single-crystal X-ray structures for both adducts **2** and **4** have been obtained.

Nearly 50 years after its discovery, erythromycin A remains one of the most widely used antibiotics in the world.[1] Extensive research over the decades in search of evermore potent compounds has led to considerable knowledge of these complex molecules. Recently, a significantly more potent subclass of macrolide antibiotics based on the erythromycin skeleton has emerged. Termed ketolides, they are characterized by the hydrolysis of the cladinose sugar followed by subsequent oxidation of the free hydroxyl to a C-3-keto moiety.[2] Abbott's ketolide clinical candidate, ABT-773 (**1**), is additionally substituted at the C-6-position with a *trans*-2-propenyl-3-(3-quinoline) (PQ) linkage and bridged across the C-11 and C-12 positions with a cyclic carbamate (see Figure 1).[3,4]

We wish to report here the discovery of a previously unknown reaction of ketolides. When ABT-773 (**1**) is heated in the presence of aqueous alcohols, cycloadduct

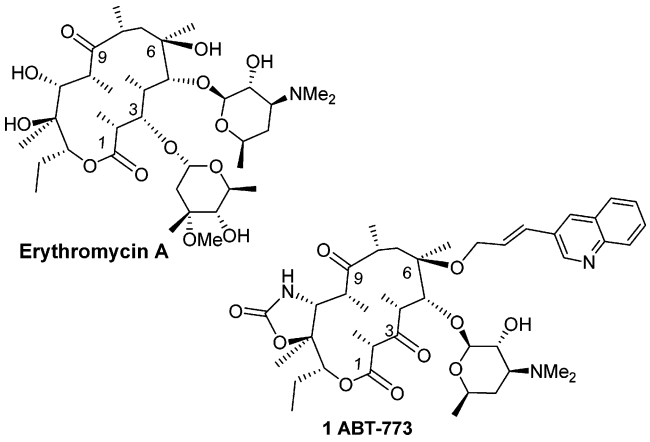
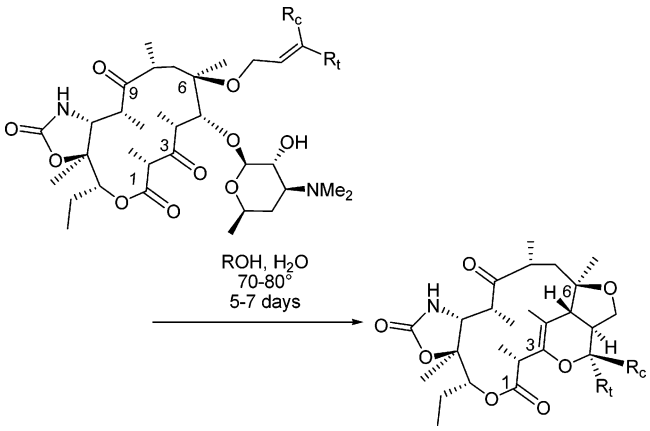


FIGURE 1. Structures of macrolide antibiotics.

TABLE 1. Intramolecular Hetero-Diels–Alder Cycloaddition



entry	starting material	R_c	R_t	adduct	yield (%)[a]
1	ABT-773 (**1**)	H	3-quinolyl	**2**	26[a]
2	*cis*-ABT-773 (**3**)	3-quinolyl	H	**4**	15[b]

[a] Typical isolated yields range from 20% to 50%. [b] The reduced yield here reflects difficulties in isolation of this compound.

2 forms (see Table 1).[5] Adduct **2** represents a new structure in the macrolide series. Adducts arising from both the cis and trans isomers of ABT-773 have been isolated and characterized with single-crystal X-ray structures (see Table 1).

From an operational standpoint, the reaction conditions are remarkably simple. Although other solvents were examined, mixtures of alcohols and water are the preferred reaction media. The composition may vary from 20% to 80% water with little impact on the reaction outcome. A variety of alcohols may be employed (MeOH, EtOH, *i*-PrOH, and *t*-BuOH).

(1) Flynn, E. H.; Sigal, M. V., Jr.; Wiley: P. F.; Gerzon, K. *J. Am. Chem. Soc.* **1954**, *76*, 3121.

(2) For an excellent review of ketolide chemistry, see: Ma, Z.; Nemoto, P. A. *Curr. Med. Chem.: Anti-Infect. Agents* **2002**, *1* (1), 15.

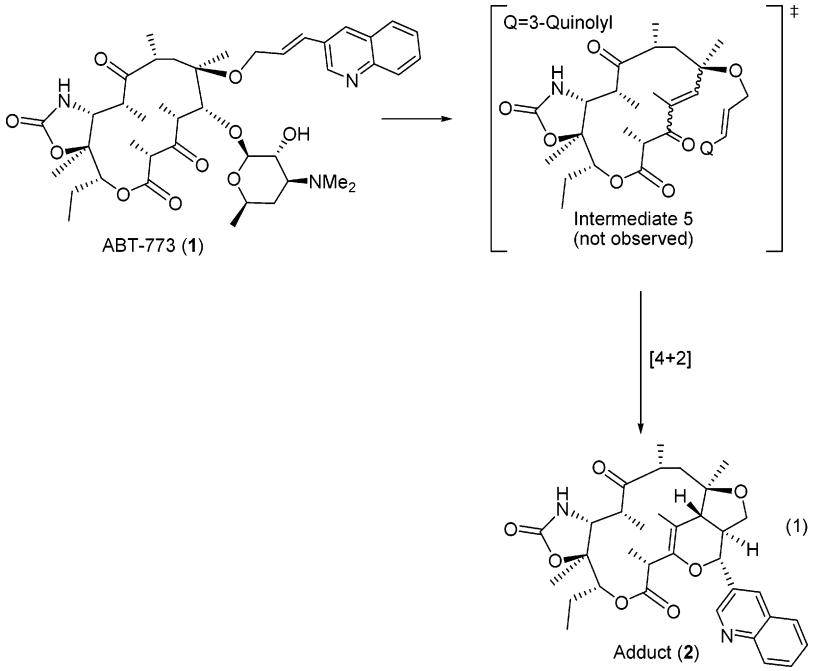
(3) (a) Or, Y. S.; Clark, R. F.; Wang, S.; Chu, D. T. W.; Nilius, A. M.; Flamm, R. K.; Mitten, M.; Ewing, P.; Alder, J.; Ma, Z. *J. Med. Chem.* **2000**, *43* (6), 1045. (b) Or, Y. S.; Ma, Z.; Clark, R. F.; Chu D. T.; Plattner, J. J.; Griesgraber, G. U.S. Patent 5,866,549, 1999.

(4) Resek, J. E.; Wang, X. C.; Bhatia, A. V. *Curr. Opin. Drug Discovery Dev.* **2000**, *3* (6), 807–817.

(5) Preliminary discussion of this reaction has been presented: Stoner, E. J.; Rehm, T.; Allen, M. S.; Bhatia, A. V.; Cirovic, M.; Henry, R.; Hollis, S.; Keyes, R.; Kristensen, E.; Marsden, I.; Narayanan, B. A.; Peterson, P.; Shiroor, S.; Stewart, K.; Tien, J. J.-H. Formation of an Unusual Impurity from ABT-773. In *Abstracts of Meetings*; 224th National Meeting of the American Chemical Society, Boston, MA.; American Chemical Society: Washington, DC, 2002.

10.1021/jo0500131 CCC: $30.25
Published on Web 03/22/2005

SCHEME 1. Proposed Mechanism for the Formation of Cycloadducts



Optimally, the reaction is performed at 70−75 °C. Reactions carried out below 60 °C proceed quite slowly, while decomposition of both starting materials and products is greatly accelerated when reactions are carried out above 85 °C.

One reaction parameter of particular interest is *apparent* solution pH.[6] Under acidic conditions (pH <7), the reaction does not proceed perceptibly, while increasing solution basicity greatly accelerates the rate of the conversion. Above pH 12, however, decomposition of products **2** and **4** is rapid and yields decrease. The optimal range has been demonstrated experimentally to be from pH 9 to 11.[7,8] At present, yields for this process appear to be limited to 20−50% due to the stated instabilities of both products and starting materials to the reaction conditions.

These experimental observations suggest a probable mechanism. It is proposed that initial enolization of the 1,3-dione system induces β-elimination of the desosamine sugar, affording putative intermediate enone **5**. Enone **5** would then react with the olefin of the 6-*O*-propenylquinoline moiety through an intramolecular hetero-Diels−Alder reaction affording cycloadduct **2** as shown in Scheme 1. We have been unable to isolate or trap enone **5** to date. However, exposure of **1** to MeOD and D_2O under the reaction conditions affords **2** deuterated at the C-2 and C-8 positions, verifying that enolization does indeed occur.

Adducts resulting from the isomerization of the C-2 methyl have not been observed despite the described likely intermediacy of enolic and dienolic structures. Under these basic reaction conditions, the C-2 position is likely to undergo equilibration, eventually returning to the most stable conformation. Conformationally driven stereocontrol at the C-10 position for a number of erythromycin analogues has been documented.[4,9]

This cycloaddition should be stereospecific with respect to olefin geometry.[10] To test this experimentally, adduct **3** (*cis*-propenylquinoline ABT-773) was prepared from **1** by photolytic isomerization. While we have been unable to isolate pure **3**, we were able to obtain samples comprised solely of **1** and **3** with the desired cis compound present in levels as high as 68%. By blending, varying ratios of **1**:**3** could be obtained. In a set of control experiments, these mixtures were exposed to the reaction conditions (1:1 water:*i*-PrOH, 70 °C), monitored over 4 days by HPLC, and the ratios of starting materials and products determined.[11] Representative data from these experiments are shown in Table 2.

It was demonstrated that **1** reacts exclusively to afford **2** (Table 2, entry C). By inference (Table 2, entries A and B), **3** reacts to afford exclusively **4**. This type of selectivity is highly indicative of a pericyclic reaction. Furthermore, control experiments demonstrate that neither the start-

(6) Termed apparent due to the high organic content of the reaction mixtures. Typically measured with an electronic probe.

(7) In a 1:1 mixture of *i*-PrOH and water, a dilute solution of **1** registers an apparent pH 10.5 and no adjustment is necessary.

(8) Additives such as tertiary amines and basic salts (NaOH, $NaHCO_3$) can afford rate acceleration to the reaction.

(9) (a) Plata, D. J.; Leanna, M. R.; Rasmussen, M.; McLaughlin, M. A.; Condon, S. L.; Kerdesky, F. A. J.; King, S. A.; Peterson, M. J.; Stoner, E. J.; Wittenberger, S. J. *Tetrahedron* **2004**, *60*, 10171. (b) Baker, W. R.; Clark, J. D.; Stephens, R. L.; Ram, R. N.; Kim, K. K. *J. Org. Chem.* **1988**, *53*, 2340.

(10) (a) For a review of the Hetero-Diels−Alder reaction see: Boger, D. L.; Weinreb, S. M. In *Hetero Diels−Alder Methodology in Organic Synthesis*; Wasserman, H. H., Ed.; Academic Press: New York, 1987. (b) For a review of the Intramolecular Diels−Alder reaction see: Ciganek, E. In *Organic Reactions*; Dauben, W. G., Ed.; Wiley: New York, 1984; Vol. 32, pp 1−374. (c) For an excellent recent review of the Hetero-Diels−Alder reaction see: Tietze, L. F.; Kettschau, G. *Top. Curr. Chem.* **1997**, *187*, 1.

(11) These are weight-to-weight ratios. Pure samples of **1**, **2**, and **4** were available for comparison. The response factor of **3** was assumed to be identical with that of **1** at 220 nm. This assumption could be verified by measuring the response of the various **1**:**3** mixtures of known weight.

TABLE 2. Assessment of the Stereospecificity of the Reaction

entry	day(s)	ratio of starting material (**1**:**3**)[a]	ratio of products (**2**:**4**)[a]	conversion (PA %)[b]
A	0	35:65	N/A	0
	2	32:68	31:69	31.1
	3	33:67	30:70	45.5
	4	32:68	29:71	55.4
B	0	78:22	N/A	0
	2	79:21	77:23	34.7
	3	77:23	75:25	47.4
	4	75:26	74:26	57.2
C	0	100:0[c]	N/A	0
	4	100:0	100:0	58.0

[a] Ratios (weight to weight) were determined by HPLC, using potency standards [b] Ratios (peak area%) of all products to the total of products and starting materials. [c] Pure trans adduct.

ing materials (**1** and **3**) nor the products (**2** and **4**) are interconvertable under the reaction conditions.

Further support of this mechanistic proposal comes from molecular modeling. The solid-state crystal structure of ABT-773 (**1**) is known. It exhibits the typical macrocyclic ring conformation observed previously in other erythromycin analogues.[12] In this structure, the 6-*O*-PQ moiety (the heterodienophile) lies directly over the plane of the macrocyclic ring.[13]

The conformation of the molecule would be expected to change significantly during formation of enone intermediate **5** as two sp^3-hybridized ring centers convert to sp^2-hybridized centers in the immediate proximity of the 6-*O*-PQ group. To replicate this, we created energy-minimized molecular models to assess possible reaction vectors between the olefin of the 6-*O*-PQ group and the enone. Using the crystal structure of ABT-773 as a template, a model of the transition state was created. The enone was created by eliminating two hydrogens, and torsion angles of the 6-*O*-PQ group were adjusted until the olefin was within reaction distance of the enone. The forming C−C and C−O bonds were constrained to be 2.0 Å distance (shown as dotted lines in Figure 2. The final structure was created by an energy minimization, using the CFF force field calculation with the InsightII software (Accelrys, San Francisco CA).

It is noteworthy that no unusual bond deformations, angles, or side chain conformations were required to generate this model. The proposed transition state involving the requisite cisoid enone and corresponding heterodienophile can be produced without significant interactions and gives acceptable attack angles to yield adduct **2**. The molecular mechanics method employed here is a rough approximation to the quantum mechanical analysis required for a detailed understanding of the bonding of atoms directly involved in the cycloaddition. While this model does not prove that a pericyclic reaction occurs, a reasonable 3D model showing that such a reaction is possible can be achieved with only a very simple constrained energy analysis. This model also fits nicely with the single-crystal X-ray structures we obtained for both adducts **2** and **4**.

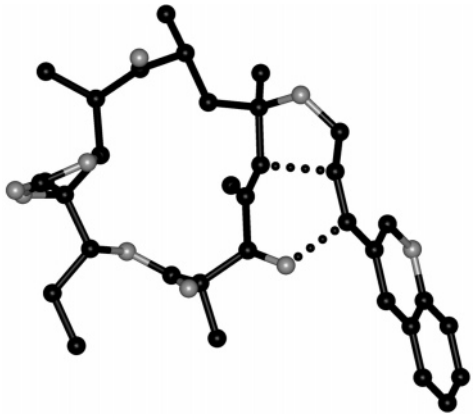


FIGURE 2. Proposed transition state for cyclization.

TABLE 3. In Vitro Antibacterial Activity of Selected Compounds (MIC)

bacterial strain	MIC (μg/mL) adduct **2**	ABT-773 (**1**)	erythromycin
S. aureus NCTC 10649	>128	0.03	0.25
S. pyogenes EES61	>128	≤0.008	≤0.008
S. pneumoniae ATCC 6303	>128	≤0.008	0.015
M. catarrhalis 2604	>128	0.06	0.25
H. influenzae 1435	>128	1	1
E. coli Juhl	>128	32	32

New structures, like **2** and **4**, based on the erythromycin skeleton are of keen interest to researchers in light of the continuing search for compounds with improved antibacterial activity.[15] Therefore, with a synthetic procedure and a supported mechanism in hand, our attention turned toward exploring the biological properties of our newly generated compounds (see Table 3). The bacteria examined are representative macrolide-susceptible respiratory tract bacterial strains. Also shown is *Escherichia coli*, which is included as an example of a gram-negative enterobacteriaceae.[16] The minimum inhibitory concentration (MIC) data for the parent structure erythromycin A, ABT-773 (**1**), and its corresponding cyclization adduct **2** are shown. Unfortunately, adduct **2** seems to be devoid of antibacterial properties.

In summary, we have described a new class of compounds formed by a previously unknown reaction of erythonolides. The compounds appear to derive from an intramolecular hetero-Diels−Alder reaction, a reaction that to our knowledge has not been reported for any erythronolides. Our mechanistic assertion is supported by both experimental and computational evidence. Although adduct **2** does not appear to exhibit the activity of its progenitor, this work may lead to the development of new antibiotic candidates.

(12) (a) Ma, Z.; Leping, L.; Rupp, M.; Zhang, S.; Zhang, X. *Org. Lett.* **2002**, *4* (6), 987. (b) Or, Y. S.; Clark, R. F.; Wang, S.; Chu D. T. W.; Nilius, A. M.; Flamm, R. K.; Mitten, M.; Ewing, P.; Alder. J.; Ma, Z. *J. Med. Chem.* **2000**, *43*, 1045.

(13) This is additionally evidenced by chemical shift differences in the ^{1}H NMR spectrum between the *cis*- and *trans*-olefin isomers of ABT-773 (see Supporting Information for discussion).

(14) Ortep representations are in the Supporting Information.

(15) (a) Ball, P.; Baquero, F.; Cars, O.; File, T.; Garau, J.; Klugman, K.; Low, D. E.; Rubinstein, E.; Wise, R. *J. Antimicrob. Chem.* **2002**, *49* (1), 3. (b) Blondeau, J. M.; DeCarolis, E.; Metzler, K. L.; Hansen, G. T. *Expert Opin. Invest. Drugs* **2002**, *11* (2), 189.

(16) MICs were determined by the broth microdilution method as specified by the National Committee for Clinical Laboratory Standards. Strains were either clinical isolates or from reference culture collections.

Acknowledgment. The authors thank Drs. Ashok Bhatia, James Jien-Heh Tien, Eric Kristensen, Angela Nilius, Steve Wittenberger, B.A. Narayanan, Momir Cirovic, and Matthew J. Peterson for valuable discussions and contributions.

Supporting Information Available: Experimental procedures and spectral data for compounds **2**, **3**, and **4**, as well as X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0500131