Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1321–1326

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF O-METHYL DERIVATIVES OF AZALIDE ANTIBIOTICS: II. 6-OME DERIVATIVES VIA CLARITHROMYCIN

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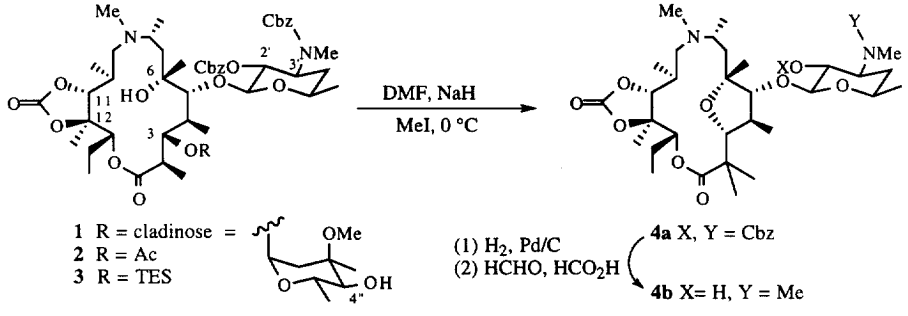
Received 24 February 1998; accepted 20 April 1998

Abstract: Direct O-methylation of various derivatives of 9-deoxo-8a- and 9a-aza-8a-homo-erythromycin (2',3'-bis-Cbz protected) gives 6-OMe derivatives only in a small number of special cases. The 6-OMe-azalides can, however, be synthesized beginning from clarithromycin.

Of all the O-methyl derivatives of the azalide antibiotics, interest in the 6-OMe derivatives is probably the keenest, perhaps on account of the great commercial success of clarithromycin[1] (**14**, the 6-OMe derivative of erythromycin.) The synthesis of the 6-OMe derivative[2] of azithromycin[3] (**20**) by direct methylation of the 2',3'-bisCbz derivative has been reported, but in the course of our investigation we discovered that this structural assignment was incorrect and that the compound in question is actually the 12-OMe derivative.[4] Thus, the preparation of the 6-OMe derivatives of azithromycin, and of its equipotent isomer 9-deoxo-8a-aza-8a-homoerythromycin[5] (**5**), remained outstanding problems. We have found that neither azithromycin nor the 8a-azalide (**5**), protected in the usual way as the 2',3'-bisCbz derivative, undergoes 6-O-methylation under the standard conditions (e.g., DMF/NaH/MeI), however vigorously they are employed.[4] Herewith, we report the results of our attempts to directly 6-O-methylate various derivatives of the 8a-azalide (**5**), and finally, our successful preparation of the 6-OMe-8a- and 9a-azalides from clarithromycin.

As reported,[4] direct O-methylation (DMF/NaH/MeI, 0 °C) of the 2',3'-bisCbz-8a-azalide gives the 4",11,12-tri-OMe derivative as the terminal methylation product: all attempts to further methylate it by extending the reaction time or raising the temperature have resulted in decomposition by loss of cladinose. A naive inspection of the 2-D structure fails to reveal why the 12-OH should be easier to O-methylate than the 6-OH: they are both tertiary hydroxy groups located on the ring nucleus. We thus concluded that the unreactivity of the 6-OH is the result of obscure conformational issues, and embarked on a program to attempt to O-methylate a variety of 8a-azalide derivatives, which might be assumed to adopt a range of conformations. We hoped in this way to find a derivative which would efficiently undergo 6-O-methylation.

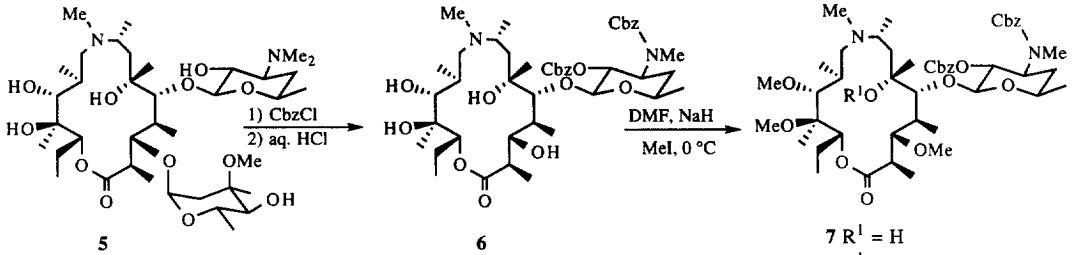


To this end, we subjected the 11,12-carbonate derivative (**1**) to the methylation conditions, and saw very rapid and efficient conversion of the starting material to a single product, which was not the 6-OMe derivative, but proved instead to possess the tetrahydrofuranyl structure (**4**). We postulate that this compound is formed by initial elimination of cladinose to form an ene-ester, followed by intramolecular Michael addition of the 6-OH to give the ester enolate, which is methylated at the 2-position to give the final product. Thus,

0960-894X/98/$19.00
PII: S0960-894X(98)00227-3

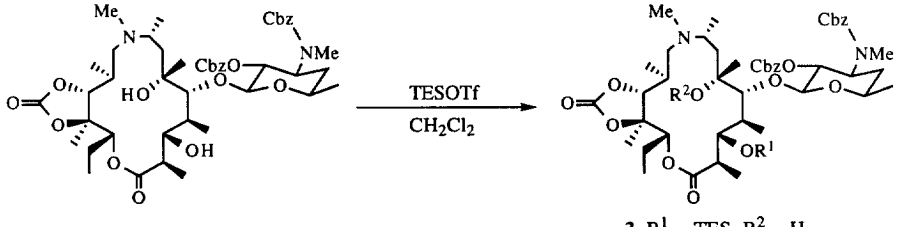
introduction of the carbonate appears to confer a conformational bias, not to 6-O-methylation, but instead to elimination of the cladinose (which is much more facile here than in the compound lacking the carbonate.)

We next investigated the methylation of the des-cladinosyl platform **6**, which bears a 3-OH function. We hoped that this alteration would make elimination across the C2-C3 bond less facile, and thus allow harsher conditions to be employed. Further, removal of the bulky cladinose substituent was expected to be accompanied by some conformational change, hopefully favorable to our ends. Subjection of this starting material to a rather harsh version of the standard methylation conditions resulted in two products: the 3,11,12-triOMe derivative **7** and the 3,6,11,12-tetraOMe derivative **8** as a 1:1 mix (no attempts were made to optimize formation of the tetramethoxylated product). We therefore succeeded in a limited sense: we saw for the first time 6-O-methylation of an 8a-azalide, but only as the last stage in a rather severe permethylation which rendered subsequent reglycosylation impossible.



5 **6** **7** R^1 = H; **8** R^1 = Me

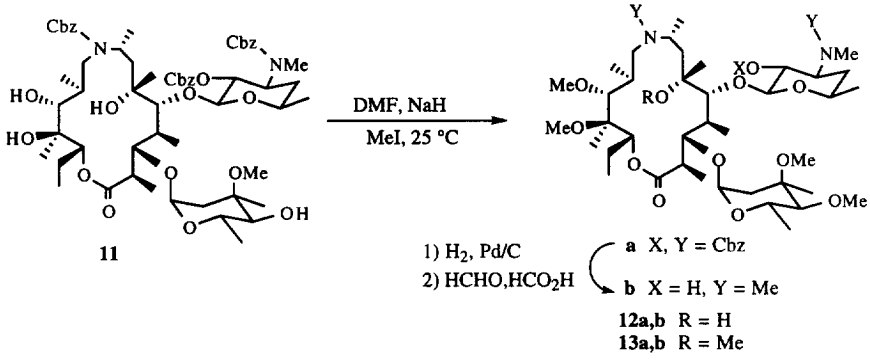
Working from the hypothesis that removal of the bulky cladinose allows for a conformational change which permits 6-O-methylation, we determined to attempt methylation of a descladinosyl derivative with protected 3-, 11-, and 12-OH's. Removal of the protecting groups and reglycosylation might then constitute a synthesis of the 6-OMe-8a-azalide. Carbonate was chosen as the protecting group for the 11- and 12-OH's, while a variety of protecting groups for the 3-OH were explored. Protection of the 3-OH as acetate, followed by subjection of this material to the methylation conditions, predictably led to formation of **4**. We next experimented with silylation as a means of protecting the 3-OH while trying to minimize its leaving group ability. We found that subjecting **9** to TESOTf in CH_2Cl_2 resulted in fast silylation of the 3-OH to give **3** and, interestingly, slow production of the 3,6-diOTES compound (**10**). Considering the bulk of the TES group, this suggested that simple steric inaccessibility was an incomplete explanation for the unreactivity of the 6-OH under the methylation conditions. In the key experiment, we were disappointed to find that subjecting the 3-OTES derivative **3** to the methylation conditions caused elimination of the OTES group to again produce the tetrahydrofuranyl derivative **4**.



9 **3** R^1 = TES, R^2 = H; **10** R^1, R^2 = TES

Finally, we turned our attention to the 2',3',8a-trisCbz derivative (**11**). We hoped that introduction of a Cbz protecting group onto the ring nitrogen might induce a substantial alteration in the conformation, both by disrupting any intramolecular hydrogen bond to the ring nitrogen, and also by opening the C-N-C bond angle. We hoped such conformational alteration might expose the 6-OH to methylation. Unexpectedly, this substrate

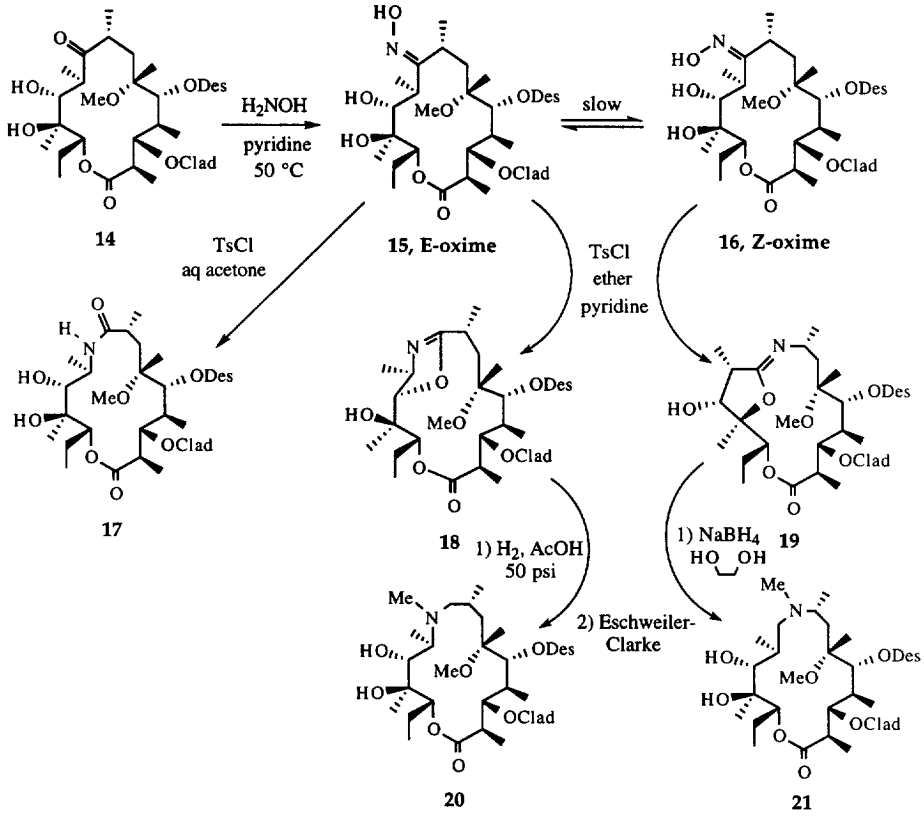
proved to be more durable than the 2'3'-bisCbz-8a-Me version: the methylation reaction could be conducted at room temperature without rapid elimination of cladinose. Under a vigorous set of methylation conditions (5 equiv NaH, 32 equiv MeI, DMF, rt, 24 h), the 4",6,11,12-tetraOMe compound (**13a**)[6] was in fact produced, but in low yield (15%, the remainder being trimethoxylated product **12a** and products from loss of cladinose.) It is unclear whether the 6-O-methylation is due to a favorable conformational bias, or simply to the more vigorous reaction conditions tolerated by the system. While this procedure allowed for the synthesis of a 6-OMe derivative of the fully glycosylated nucleus by direct methoxylation, it was once again in the necessary context of permethylation, and not readily adaptable to the synthesis of the mono-6-OMe derivative.



Having failed to accomplish direct selective 6-methoxylation of the azalide nucleus, we resolved to attempt the synthesis of the 6-OMe-azalides from clarithromycin. We envisioned a sequence that parallelled the synthesis of the 6-OH-azalides from erythromycin, but we anticipated several special difficulties at the outset. With the 6-OH blocked, the task of capturing the cationic Beckmann intermediate intramolecularly (to form an iminoether) would fall to the 11- and 12-OH.[7] We further recognized that the base-catalyzed isomerization of the *E*-oxime to the *Z*-oxime,[5] which is the critical synthetic fork leading to the 8a-azalides, was likely to fail in the 6-OMe system, depending as it does on a felicitous arrangement of the proper functional groups.

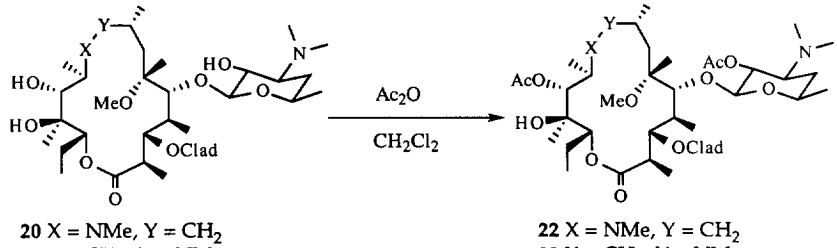
The first step of the sequence, the reaction of clarithromycin (**14**) with hydroxylamine-hydrochloride in pyridine, was much slower than the analogous reaction with erythromycin, and required elevated temperatures and an extended reaction time. Analysis by proton NMR suggested stereospecific formation of the *E*-oxime (**15**). When this compound was subjected to the conditions reported for the conversion of erythromycin-*E*-oxime to the 6,9-iminoether (TsCl, $NaHCO_3$, aq. acetone), clean conversion instead to the macrolactam **17** was observed. Reaction in pyridine, on the contrary, gave clean conversion to the desired 9,11-iminoether (**18**).[8] This compound was hydrogenated on a Parr shaker to provide 6-OMe-azathromycin,[9] which was subjected to the usual Eschweiler-Clarke methylation of the ring nitrogen to provide 6-OMe-azithromycin (**20**).[6] This represents, to the best of our knowledge, the first reported synthesis of this compound.

We next focussed on the synthesis of the 6-OMe-8a-azalide. Our above-mentioned skepticism proved to be well-founded when we observed no isomerization of the *E*-oxime of clarithromycin upon submitting it to the basic conditions which had been shown to convert the erythromycin-*E*-oxime to *Z*. However, a small amount of a by-product, slightly lower R_f by TLC (95:5 EtOAc/Et_3N eluant), had been observed in some oxime-formation reactions (H_2NOH-HCl, pyridine, 45 °C) which had been allowed extended reaction times (on the order of days). Repeated chromatography supplied a pure sample of this material, which was identified as the *Z*-oxime (**16**). We soon discovered that the *Z*-oxime, in contrast to the *E*-isomer, had limited solubility in methylene chloride, which allowed for a relatively easy purification of this minor isomer by crystallization. In this manner we determined that the equilibrium mixture consisted of about 10% of the *Z*-isomer.[10] Resubmission of the purified *E*-isomer to the reaction conditions served to reestablish the equilibrium mixture, from which could be crystallized the newly formed *Z*-oxime. It can be seen that, by repetition in this manner, a sample of clarithromycin-*E*-oxime could be essentially completely (if laboriously) isomerized. In practice, a small number of iterations on multigram scale provided useful amounts of material.



With clarithromycin-Z-oxime in hand, the synthesis of the 6-OMe-8a-azalide was accomplished in a relatively straightforward manner, following a sequence similar to that reported for the synthesis of the 8a-azalide from erythromycin. Thus, the Beckmann gave the 9,12-iminoether (**19**) as the exclusive product, which compound was reduced using sodium borohydride in ethylene glycol. Eschweiler-Clarke methylation of the ring nitrogen furnished the desired 6-OMe-8a-azalide (**21**)[6].

An interesting consequence of 6-O-methylation in both the 8a- and 9a-aza platforms is the increased susceptibility of the 11-OH to acetylation under extremely mild conditions (Ac_2O, CH_2Cl_2). The 6-OH azalides, like erythromycin, are acetylated only on the 2'-OH under these conditions, as a result of intramolecular general base catalysis by the 3'-NMe_2. Our assumption is that the conformational effect of 6-O-methylation in the azalides is such that the ring nitrogen is now able to accept a hydrogen bond from the 11-OH, and therefore provide similar catalysis at this position, which results in the 2',11-diOAc derivatives (**22** & **23**).



20 X = NMe, Y = CH_2
21 X = CH_2, Y = NMe

22 X = NMe, Y = CH_2
23 X = CH_2, Y = NMe

Examination of Table 1 shows that the effect of 6-O-methylation is modestly deleterious in both platforms (8a-azalide (**5**) vs. **21**, azithromycin (**24**) vs. **20**). Comparison of the 6-OMe-8a-azalide (**21**) with the 4"-OMe-derivative (**25**)[4] shows that methylation of the 6-OH is slightly better tolerated. The 4",6,11,12-tetra-OMe 8a-azalide (**13b**) has approximately the same activity as the 4", 11,12-tri-OMe derivative (**12b**). In the 9a-aza platform, the 6-OMe derivative (**20**) is somewhat less good overall than either the 11-OMe isomer (**26**)[2] or the 12-OMe isomer (**27**)[2,4].

Table 1 MIC's versus selected organisms (sensitive strains)

		MIC's (mg/mL)								
strain		**5**	**21**	**25**	**12b**	**13b**	**24**	**20**	**26**	**27**
S. pneumo	(MB3957)	<0.06	0.5	0.5	4	4	<0.06	0.5	0.03	0.03
S. pyogenes	(MB2874)	<0.06	0.06	0.03	0.5	0.5	0.03	0.12	0.03	0.03
S. aureus	(MB2865)	0.3	2	8	32	32	0.5	2	1	1
E. faecalis	(MB5407)	2	16	8	32	32	4	8	8	4
E. faecium	(MB5516)	0.125	1	2	8	8	0.25	1	0.5	0.25
B. subtilis	(MB5586)	0.3	1	4	2	8	1	1	1	-

In conclusion, we have shown that patterns of O-methylation of the 8a- and 9a-azalides, while grossly resembling each other, differ substantially from that exhibited by erythromycin. Specifically, the azalides undergo facile methylation of the 12-OH while methylation of the 6-OH is not observed, whereas with erythromycin, the 6-OH (along with the 11-OH) is first to be methylated.[1b] Critical to reaching this conclusion was the unifying discovery that the compound reported in the literature[2] as 6-OMe-azithromycin is actually 12-OMe-azithromycin[4]. A successful preparation of the 6-OMe derivatives of both the 8a- and 9a-azalides from clarithromycin has been demonstrated.

References and Notes

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6. Complete proton and carbon NMR assignments for the new OMe azalide derivatives **12b, 20** and **21** were made using a combination of two-dimensional NMR techniques which allowed for the complete and unambiguous assignment of the carbon and proton spectra: COSY (proton-proton correlation) and HMQC (one bond proton-carbon correlation) together allowed for an almost complete assignment, with only the quaternary carbons (3", 6 & 12), protons on methyl groups attached to quaternary carbons (3"-Me, 6-Me & 12-Me), and methoxy protons (3"-OMe plus any introduced methoxys) remaining unassigned. The long-range HMBC experiment, which correlates protons to carbons over 2 and 3

bonds, eliminated all remaining ambiguities. First, it allowed carbons 6 and 12 to be unambiguously distinguished in the ^{13}C NMR spectrum. Next, we found that in every case a very strong coupling is seen from the protons on a methoxy group to the carbon bearing that methoxy group. With a complete carbon assignment, this makes it a simple matter to determine the locations of the methoxy groups. This data is summarized in the table which follows:

Table 2 NMR assignments for selected compounds

	^1H NMR (500 MHz, $CDCl_3$, 50 °C)				^{13}C NMR (125 MHz, $CDCl_3$, 50 °C)										
proton	12b	20	21	proton	12b	20	21	carbon	12b	20	21	carbon	12b	20	21
2	2.76	2.88	2.64	6′	1.28	1.23	1.23	1	176.5	176.8	177.0	1″	96.3	95.1	95.5
3	4.26	4.00	4.16	1″	5.01	4.97	5.00	2	46.5	45.1	45.5	2″	35.3	35.0	34.8
4	1.99	2.10	1.65	2″a	2.48	2.36	2.37	3	77.3	79.1	77.3	3″	74.5	72.8	72.7
5	3.77	3.77	3.76	2″b	1.53	1.61	1.53	4	41.5	39.7	41.4	4″	89.0	77.8	78.0
7a	1.84	1.96	1.78	4″	2.66	3.03	3.03	5	78.8	79.9	78.9	5″	64.4	65.7	65.6
7b	1.52	1.20	1.49	5″	4.32	4.09	4.09	6	79.0	79.9	79.5	6″	17.8	18.2	18.1
8	2.95	1.79	2.92	6″	1.29	2.32	1.31	7	43.2	37.9	40.8	2-Me	14.8	15.7	14.5
9a	2.46	2.52	2.39	2-Me	1.24	1.25	1.22	8	54.7	27.8	55.5	4-Me	9.9	9.2	9.7
9b	2.24	2.09	2.39	4-Me	1.14	1.11	1.02	9	64.1	63.2	61.3	6-Me	29.0	21.1	21.0
10	1.87	2.75	1.90	6-Me	1.28	1.37	1.32	10	31.8	61.0	32.0	8-Me	14.1	22.0	13.7
11	3.50	3.60	3.72	8-Me	0.95	0.94	0.80	11	83.8	75.1	70.0	10-Me	12.1	8.8	13.3
13	5.44	4.89	5.00	10-Me	0.94	1.05	0.99	12	81.8	-	76.1	12-Me	15.9	16.2	16.1
14a	1.85	1.92	1.93	12-Me	1.10	1.13	1.09	13	75.8	78.0	77.3	3″-Me	21.1	17.9	21.1
14b	1.55	1.55	1.50	3″-Me	1.27	1.27	1.25	14	22.2	21.6	22.0	NMe2	39.8	40.2	40.0
15	0.91	0.92	0.92	NMe2	2.30	2.31	2.30	15	10.5	10.8	11.0	NMe	33.1	41.1	35.0
1′	4.45	4.49	4.51	NMe	2.07	2.32	2.10	1′	103.0	102.7	102.5	3″OMe	49.3	49.0	49.0
2′	3.18	3.22	3.19	3″OMe	3.34	3.35	3.34	2′	70.8	71.2	71.1	4″OMe	61.4	-	-
3′	2.56	2.46	2.48	4″OMe	3.56	-	-	3′	64.7	65.4	65.2	6OMe	49.7	50.3	50.5
4′a	1.64	1.65	1.65	6OMe	3.20	3.28	3.27	4′	28.5	29.0	29.0	11OMe	61.8	-	-
4′b	1.18	1.23	1.20	11OMe	3.52	-	-	5′	68.3	68.5	67.7	12OMe	52.2	-	-
5′	3.58	3.53	3.54	12OMe	3.35	-	-	6′	20.8	21.1	21.1				

7. The Beckmann reaction of erythromycin-*E*-oxime was initially reported (ref 3a) to give the 6,9-iminoether exclusively (via trapping of the cationic intermediate by the 6-OH.) Although a subsequent paper (ref 3c) reported a low temperature variation that produced a mixture of the 6,9- and 9,11-iminoethers, it was apparent that trapping by the 6-OH was most facile, and the consequences of blocking it were uncertain.
8. This is again contrary to the report regarding erythromycin-*E*-oxime, where this set of conditions gave formation of the macrolactam. Our results do, however, parallel those seen for the reaction of erythromycin-*Z*-oxime (ref 5).
9. Our results here parallel Yang's observation (ref 3c) that the erythromycin-derived 9,11-iminoether is susceptible to lower pressure hydrogenation (50 psi) than that required for hydrogenation of the isomeric 6,9-iminoether (1500 psi).
10. That the 90/10 mix of *E/Z* oxime is in fact the equilibrium ratio was verified by submitting a sample of purified *Z*-oxime to the reaction conditions, and observing after several days the same 90/10 mix of *E/Z*.