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Phosphonoxins III: Synthesis of α-Aminophosphonate Analogs of Antifungal Polyoxins with Anti-*Giardia* Activity

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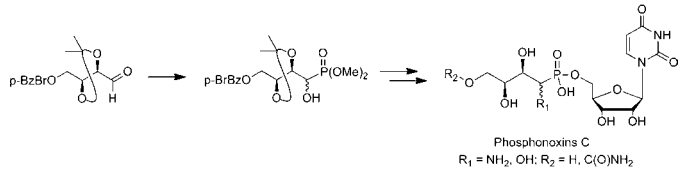
ABSTRACT



A synthesis of α-aminophosphonate analogs of polyoxins, termed phosphonoxin C1, C2, and C3, has been achieved. The key step was the addition of lithium dimethyl phosphite to the aldehyde of a protected threose derivative. α-Hydroxyphosphonate analogs C4 and C5 were also obtained by taking advantage of an unprecedented conversion of an azide to hydroxyl during treatment with hydrogen on palladium on carbon. The resulting phosphonoxin C5 inhibited the growth of an intestinal protozoan, *Giardia lamblia*, at low micromolar concentration.

The natural products polyoxins isolated from the *Streptomyces* species are a closely related class of peptidylnucleoside antibiotics.[1] They have been shown to be potent inhibitors of many types of fungi and parasites due to their ability to disrupt cell envelope biosynthesis.[2] For example, one such parasite is *Giardia lamblia*,[3] a protozoan parasite that colonizes and replicates in the intestinal tract. It is one of the most common causes of diarrhea in the developed world and is not only restricted to humans but affects livestock and companion animals, where there are an estimated 100 million cases per year worldwide.[4] Therefore, the need for effective treatment continues to this date. Polyoxins[5] most likely act by mimicking the stucture of, and thereby competing with, the natural substrate for cell envelope biosynthesis, uridine diphosphoryl-*N*-acetylglucosamine (UDP-GlcNAc, Figure 1).[6] They have exhibited activity against many types of fungi[7] and inhibit cell wall formation in *Entamoeba*.[8] However, utility of these natural products as drugs is compromised by their poor bioavailability and metabolic instability resulting in low efficacy evidenced by

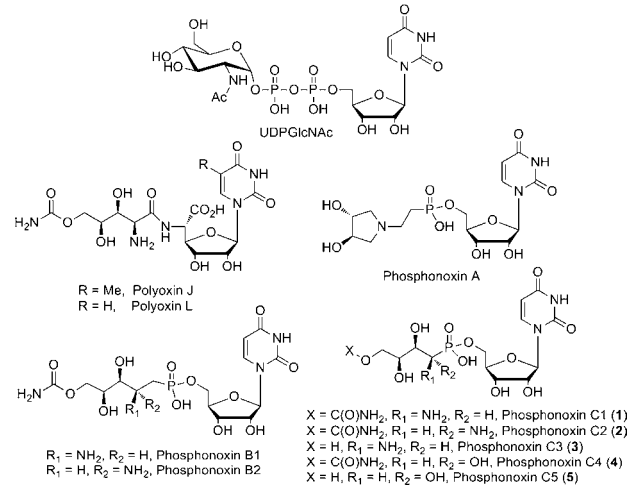


Figure 1. UDP-GlcNAc, polyoxins J and L, and phosphonoxins.

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(1) (a) Brock, T. D. *Biology of Microrganisms*; Madigan, M. T., Martinko, J. M., Dunlap, P. V., Clark, D. P., Eds.; Pearson Education Ltd: Upper Saddle River, NJ, 2003. (b) For a review on polyoxins, see: Akita, H. *Heterocycles* **2009**, *77*, 67, and references therein.

(2) (a) Debono, M.; Gordee, R. S. *Annu. Rev. Microbiol.* **1994**, *48*, 471. (b) Riuz-Herrera, J.; San-Blas, G. *Curr. Drug Targets- Infect. Disorders* **2003**, *3*, 77.

(3) Karr, C. D.; Jarroll, E. L. *Microbiology* **2004**, *150*, 1237.

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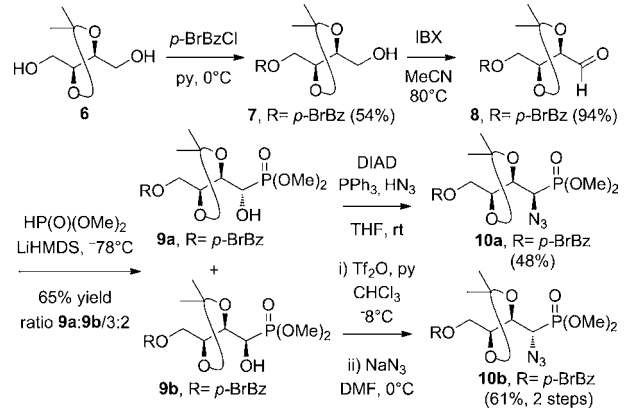
high inhibitory concentrations against fungal pathogens.[9] Over the years, many groups have synthesized polyoxin analogs with the goal of obtaining compounds that would retain the antifungal and antiparasitic activity of polyoxins but could be more suitable as drugs.[5,10]

We recently published the synthesis of a new class of polyoxin analogs, termed *phosphonoxins*, that replaced the peptide linkage to the nucleoside with a phosphonate linkage.[11,12] Phosphonates are chemically and enzymatically stable and many such derivatives are able to penetrate cells.[13] One of the phosphonoxins we synthesized (here termed phosphonoxin A, Figure 1) is a potent inhibitor of *Giardia* trophozoite growth and cyst formation *in vitro*.[11] We also synthesized phosphonoxins B1 and B2, which more closely resemble the structure of the natural polyoxins.[12]

We report here the synthesis of phosphonoxins C1 (**1**) and C2 (**2**), which differ from the phosphonoxins B only in that they do not possess the methylene spacer between the amino phosphorus groups; they are α- instead of *β*-aminophosphonates. During the course of this synthesis we also obtained α-hydroxyphosphonate analogs, phosphonoxins C4 (**4**) and C5 (**5**), by the unexpected conversion of an azide into a OH group under catalytic hydrogenation conditions. Our approach to the synthesis of the phosphonoxins C involved a similar approach to that taken to synthesize phosphonoxins B.[12]

The α-amino stereocenter would be generated by reaction of a chiral aldehyde with the anion of dimethyl phosphite[14] instead of sulfinimine with dimethyl methylphosphonate.[15] We also would use a *p*-bromo benzoyl instead of a benzyl protecting group to aid in the crystallization of intermediates for the determination of stereochemistry by X-ray crystallography. We began with the reaction of commercially available diol **6** with one equivalent of 4-BrBz-Cl in pyridine to give predominantly monobenzoylated product **7** (Scheme 1). Oxidation of alchohol **7** with IBX[16]

Scheme 1. Synthesis of α-Diazophosphonates



generated aldehyde **8**. This aldehyde was then treated with the lithium anion of dimethyl phosphite[17] to produce α-hydroxyphosphonate diastereomers **9a** and **9b** in a 3:2 ratio in 65% combined yield. These isomers were readily separated by column chromatography. Both isomers, **9a** and **9b**, were then independently converted to the corresponding azides,[18] **10a** and **10b**, via Mitsunobu substitution[19] or triflation of the hydroxyl group followed by nucleophilic displacement with azide.

The stereochemistry of **10b** was confirmed by condensation of aldehyde **8** with (*R*)-*p*-toluenesulfinamide[20,21] to produce (*R*)-sulfinimine **11** in low yield due to cleavage of the benzoate moiety (Scheme 2). This sulfinimine was then reacted with the lithium anion of dimethylphosphite to give phosphonate **12a** with high selectivity (90% de) over its diastereomer **12b**. The absolute configuration of **12a** was confirmed by X-ray crystallography.

(4) (a) Gajadhar, A. A.; Allen, J. R. *Vet. Parasitol.* **2004**, *126*, 3. (b) Ali, S. A.; Hill, D. R. *Curr. Opin. Infect. Dis.* **2003**, *16*, 453. (c) Wright, J. M.; Dunn, L. A.; Upcroft, P.; Upcroft, J. A. *Expert Opin. Drug Saf.* **2003**, *2*, 529.

(5) (a) Isono, K.; Suzuki, S. *Heterocycles* **1979**, *13*, 333. (b) Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490. (c) Isono, K. *J. Antibiot.* **1988**, *41*, 1711. (d) Zhang, D.; Miller, M. J. *Curr. Pharmac. Des.* **1999**, *5*, 73. (e) Akita, H. *Heterocycles* **2009**, *77*, 67.

(6) Surarit, R.; Gopal, P. K.; Shepherd, M. G. *J. Gen. Microbiol.* **1988**, *134*, 1723.

(7) (a) Hori, M.; Eguchi, J.; Kakild, K.; Misato, T. *J. Antibiot.* **1974**, *27*, 260. (b) Hori, M.; Kakiki, K.; Misato, T. *Agric. Biol. Chem.* **1974**, *38*, 699. (c) Calib, E. *Antimicrob. Agents Chemother.* **1991**, *35*, 170. (d) Li, R. K.; Renaldi, M. G. *Antimicrob. Agents Chemother.* **1999**, *43*, 1401.

(8) Avron, B.; Deutsch, R. K.; Mirelman, D. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 815.

(9) (a) Mehta, R. J.; Kingsbury, W. D.; Valenta, J.; Actor, P. *Antimicrob. Agents Chemother.* **1984**, *25*, 373. (b) Hori, M.; Kakiki, K.; Misato, T. *Agric. Biol. Chem.* **1974**, *38*, 698. (c) Dooday, G. W. In *The Biochemistry of Cell Walls and Membranes in Gungi*; Goosey, M., Kuhn, P., Trinci, A. P. J., Eds.; Springer-Verlag: Berlin, 1989; p 61. (d) Mitani, M.; Inoue, Y. *Antibiot.* **1968**, *21*, 492. (e) McCarthy, P. J.; Troke, P. F.; Gull, K. *J. Gen. Microbiol.* **1985**, *131*, 77540. (f) Becher, J. M.; Covert, M. L.; Shenbagamurthi, P.; Steinfeld, A. S.; Naider, F. *Antimicrob. Agents Chemother.* **1983**, *23*, 926.

(10) (a) Luo, Y.-C.; Zhang, H.-H.; Liu, Y.-Z.; Cheng, R.-L.; Xu, P. F. *Tetrahedron: Asymmetry* **2009**, *20*, 1174. (b) Plant, A.; Thompson, P.; Williams, D. M. *J. Org. Chem.* **2008**, *73*, 3714. (c) Yeager, A. R.; Finney, N. S. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 6451. (d) Obi, K.; Uda, J.-I.; Iwase, K.; Sugimoto, O.; Ebisu, H.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1451. (e) Cooper, A. B.; Desai, J.; Lovey, R. G.; Saksena, A. K.; Girijavallabhan, V. M.; Ganguly, A. K.; Loebenberg, D.; Parmegiani, R.; Cacciapuoti, A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1079. (f) Naider, F.; Shenbagamuthi, P.; Steinfeld, A. S.; Smith, H.; Boney, C.; Becker, J. M. *Antimicrob. Agents Chemother.* **1983**, *24*, 787.

(11) Suk, D.-H.; Bonnac, L.; Dykstra, C. C.; Pankiewicz, K. W.; Patterson, S. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2064. (b) Suk, D. H.; Rejman, D.; Dykstra, C. C.; Pohl, R.; Pankiewicz, K. W.; Patterson, S. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2811.

(12) Zhou, D.; Staake, M.; Patterson, S. E. *Org. Lett.* **2008**, *10*, 2179.

(13) (a) Blackburn, G. *Chem. Ind.* **1981**, 131. (b) Pankiewicz, K.; Goldstein, B. M. Inosine Monophosphate Dehydrogenase A Major Therapeutic Target. In *ACS Symposium Series No. 839*; Pankiewicz, K., Goldstein, B. M., Eds.; Oxford University Press: Cambridge, 2003; pp 1−17.

(14) (a) Davis, F.; Wu, Y.; Yan, H.; McCoull, W.; Prasad, K. *J. Org. Chem.* **2003**, *68*, 2410. (b) Davis, F.; Lee, S.; Yan, H.; Titus, D. *Org. Lett.* **2001**, *3*, 1757. (c) Davis, F.; Prasad, K. R.; Carroll, P. J. *J. Org. Chem.* **2002**, *67*, 7802.

(15) Micolajczyk, M.; Lyzwa, P.; Drabowicz, J.; Wieczorek, M.; Biaszczyk, J. *J. Chem. Soc., Chem. Commun.* **1996**, 1503.

(16) (a) Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537. (b) More, J. D.; Finney, N. S. *Org. Lett.* **2002**, *4*, 3001.

(17) Paulsen, H.; Kuehne, H. *Chem. Ber.* **1975**, *108*, 1239.

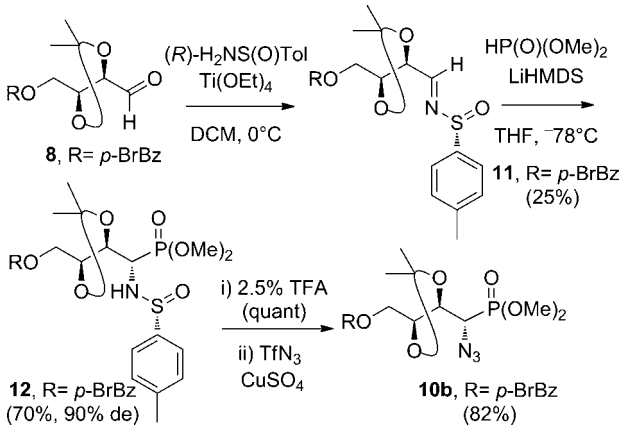
(18) CAUTION! Azides are hazardous, see: Keicher, T.; Lobbecke, S. *Lab Scale Synthesis of Azido Compounds: Safety Measures and Analysis in Organic Azides: Synthesis and Applications*; Brase, S., Banert, K., Eds.; John Wiley & Sons: Chichester, 2010; pp 1−28.

(19) Loiberner, H.; Zbiral, E. *Helv. Chim. Acta* **1976**, *59*, 2100.

(20) Davis, F. A.; Zhang, Y.; Andemichael, Y.; Fang, T.; Faneli, D. L.; Zhang, H. *J. Org. Chem.* **1999**, *64*, 1403.

(21) Liu, G.; Cogan, D. A.; Owens, T. D.; Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 1278.

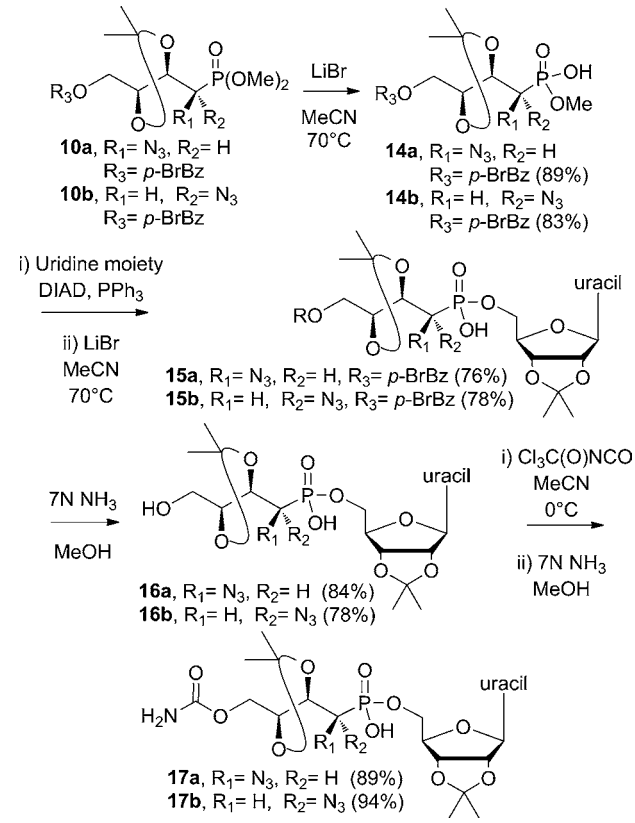
Scheme 2. α-Diazo Stereocenter Confirmation of **10b**



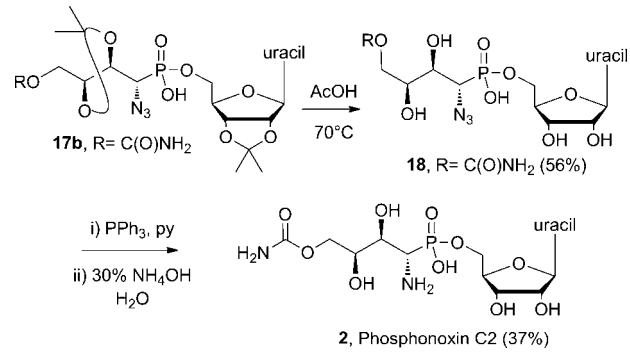
Converting sulfinamide **12**, of known stereochemistry, to amine **13** by acid catalyzed cleavage of the sulfinyl group followed by diazo transfer with TfN_3 and catalytic $CuSO_4$[22] gave an α-aminophosphonate identical in all aspects to **10b**. As the diazo transfer is known to proceed with retention of configuration,[22] the structure of **10b** was determined as depicted. The azides **10a** and **10b** were next reacted independently with LiBr at 70 °C to cleave one of the phosphonate methyls[23] giving monomethyl esters **14a** and **14b**, respectively (Scheme 3). The resulting phosphonic acids were then coupled with 2′,3′-*O*-isopropylidineuridine under Mitsunobu conditions,[24] followed by removal of the remaining phosphonate methyl by again heating in the presence of LiBr to give uridine phosphonates **15a** and **15b**. The benzoate ester was cleaved by heating at 50 °C in methanolic ammonia, resulting in **16a** and **16b**. Carbamates **17a** and **17b** were produced by treating **16a** and **16b** with trichloroacetyl isocyanate followed by ammoniolysis of the resulting trichloroacetyl group.[25] Phosphonoxin C2 was finally obtained via removal of the acetonide group of compound **17b** by heating in 80% AcOH (Scheme 4) followed by the reduction of the azide under Staudinger conditions[26] to give uridine amine **2** in 34% yield.

To avoid the low yields of the Staudinger reduction, the azide compounds **16a** and **17a** were instead reduced by hydrogenation with catalytic 5% Pd on $BaSO_4$ (Scheme 5) in methanol to give amines **19** and **21** as the major products in 72% and 48% yields, respectively. Removal of the acetonide protecting group of each by heating in 80% acetic acid in water gave phosphonoxin C3 (**3**), lacking the carbamate moiety, and phosphonoxin C1 (**1**). To our surprise, the palladium mediated azide reduction reaction also gave

Scheme 3. Uridine Phosphonoxin Intermediates



Scheme 4. Phosphonoxin C2

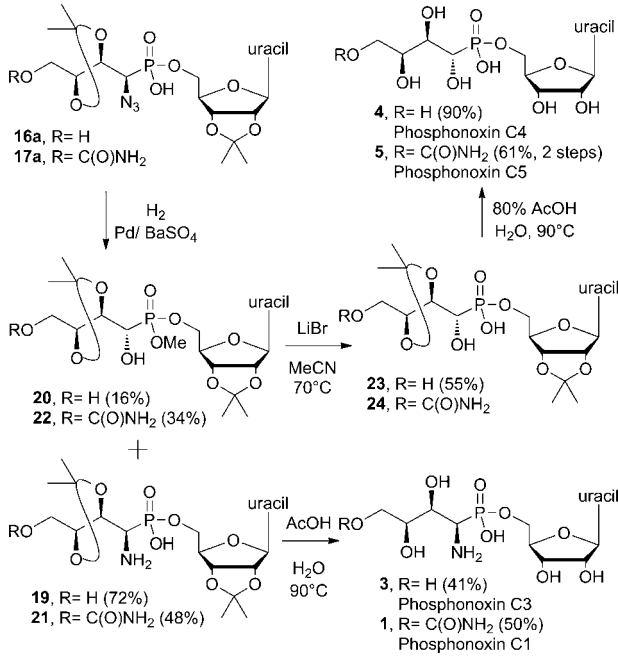


significant amounts (16% and 34% yields) of methyl α-hydroxyphosphonates **20** and **22** from **16a** and **17a**, respectively (Scheme 5). We investigated this process further and found that the reaction requires hydrogen gas to be present, as no reaction occurs with **16a** and **17a** when only the palladium catalyst is present. The identities of **20** and **22** were confirmed by cleaving the methyl ester with LiBr to obtain **23** and **24**, each with 94% diastereomeric purity, then synthesizing **23** by an alternate route from **9a** (Scheme 6).

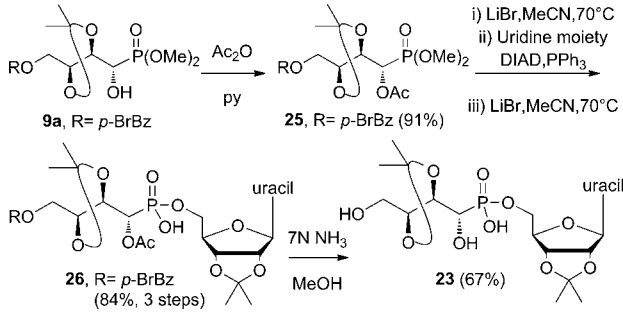
Protection of the free OH group of **9a** as an acetate group, coupling with 2′,3′-isopropylidineuridine followed by ammonia deprotection gave **23**. Compound **23** obtained via this alternate route was identical to major isomer **23** obtained from **20**.

(22) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773.
(23) Karaman, R.; Goldblum, A.; Breuer, E.; Leader, H. *J. Chem. Soc., Perkin Trans. 1* **1989**, 765.
(24) McKenna, C. E.; Kashemirov, B. A.; Rozé, C. N. *Bioorg. Chem.* **2002**, *30*, 383.
(25) (a) Murphy, C. F.; Koehler, R. E.; Webber, J. A. *Tetrahedron Lett.* **1972**, 1585. (b) Tabusa, F.; Yamada, T.; Suzuki, K.; Mukaiyama, T. *Chem. Lett.* **1984**, 405.
(26) Mungall, W. S.; Greene, G. L.; Heavner, G. A.; Letsinger, R. L. *J. Org. Chem.* **1975**, *40*, 1659.

Scheme 5. Phosphonoxins C1, C3, C4, and C5



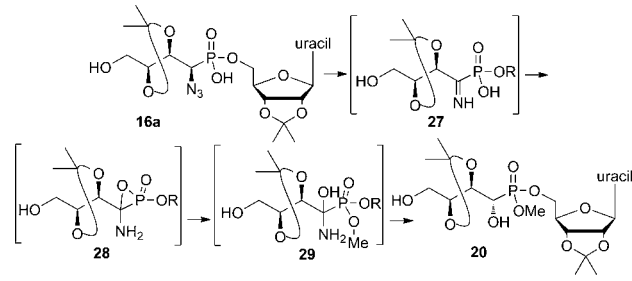
Scheme 6. α-Hydroxy Stereocenter Confirmation of **23**



Removal of the acetonide groups of **23** and **24** gave α-hydroxyphosphonate analogs, phosphonoxin C4 (**4**) and C5 (**5**) (Scheme 5). To our knowledge, this is the first example of conversion of an azide to an alcohol by catalytic hydrogenation.

In the treatment of azide **16a** with H_2 and Pd on $BaSO_4$ we propose the methyl phosphonate ester product **20** is consistent with a reactive intermediate involving the phosphonate moiety (Scheme 7). Imine intermediates such as structure **27** have been proposed for catalytic hydrogenation of azides[27] and oxaphosphiranes similar to proposed intermediate **28** have been described in other reaction pathways.[28] Attack of methanol on such an intermediate should give heminaminal **29**, which after

Scheme 7. Proposed Mechanism of Azide to Alcohol Conversion



collapse to an α-ketophosphonate and stereoselective reduction would give the observed product **20**.[29]

Table 1. Inhibition of *Giardia lamblia* Trophozoite Growth

compound	IC_{50} (μM)
Phosphonoxin C1 (**1**)	>20
Phosphonoxin C2 (**2**)	>20
Phosphonoxin C3 (**3**)	>20
Phosphonoxin C4 (**4**)	>20
Phosphonoxin C5 (**5**)	2.3

The phosphonoxins were examined for their inhibition of *Giardia lamblia* growth in culture (Table 1).[30] To our surprise, phosphonoxin C5 (**5**) obtained from the unique conversion of azide to alcohol was by far the most active, with an IC_{50} of 2.3 μM.

In conclusion, we have synthesized three novel α-aminophosphonate analogs of polyoxins, which we termed phosphonoxins C1, C2 and C3. In addition, we synthesized two α-hydroxyphosphonate analogs, C4 and C5, from the coproducts of the azide reduction, the latter of which inhibited *Giardia* trophozite growth. The scope and mechanism of the rearrangement that results in the coproducts is still under investigation. Further chemical and biological studies of the phosphonoxins and the α-hydroxyphosphonates will be described in due course.

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Supporting Information Available: Experimental procedures, spectral data for all compounds and X-ray data for compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) An, I. H.; Seong, H. R.; Ahn, K. H. *Bull. Korean Chem. Soc.* **2004**, *25*, 420.

(28) (a) Roeschenthaler, G. V.; Sauerbrey, K.; Schmutzler, R. *Chem. Ber.* **1978**, *111*, 3105. (b) Biosdon, M. T.; Barrans, J. *J. Chem. Soc., Chem. Comm.* **1988**, 615. (c) Petnehazy, I.; Clementis, G.; Jaszay, Z. M.; Toke, L.; Hall, D. C. *J. Chem. Soc., Perkin Trans. II* **1996**, 2279. (d) Savostina, L. I.; Aminova, R. M.; Mironov, V. F. *Russ. J. Gen. Chem.* **2006**, *76*, 1031.

(29) Goulioukina, N. S.; Bondarenko, G. N.; Bogdanov, A. V.; Gavrilov, K.; Beletskaya, I. P. *Eur. J. Org. Chem.* **2009**, 510.

(30) (a) Bell, C. A.; Dykstra, C. C.; Naiman, N. A.; Cory, M.; Fairley, T. A.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **1993**, *37*, 2668. (b) Das, S.; Gillin, F. D. *Exp. Parasitol.* **1996**, *83*, 19.