Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 695–698

Derivatives of pentamidine designed to target the *Leishmania* lipophosphoglycan

Kari L. Kramp,[a] Kristin DeWitt,[b] Jason W. Flora,[a] David C. Muddiman,[a,†] Kelli M. Slunt[b,*] and Todd A. Houston[a,c,*]

[a]*Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284, USA*
[b]*Department of Chemistry, University of Mary Washington, Fredericksburg, VA 22401, USA*
[c]*School of Science and Eskitis Institute, Griffith University, Nathan, QLD 4111, Australia*

Received 1 October 2004; revised 22 November 2004; accepted 22 November 2004

Abstract—The *Leishmania* lipophosphoglycan (LPG) is the most abundant cell surface glycoconjugate of a family of infectious protozoa. Pentamidine, a common drug used in the treatment of *Leishmania* infections, has been modified with boronic acids so that it might bind more selectively to the phosphodisaccharide repeating unit of the LPG. This could serve to target the drug to the protozoan surface and increase its efficacy in vivo.

Leishmaniasis is a potentially fatal disease that afflicts millions of people in tropical regions of the world.[1] The *Leishmania* protozoa that cause the disease are typically transmitted by sandflies during a bloodmeal. The parasite exists within the sandfly as an extracellular, noninfectious promastigote, but during the process of metacyclogenesis, the promastigotes cease dividing, detach from the epithelial cells of the midgut and migrate to the mouthparts of the insect.[2] These virulent metacyclic promastigotes are taken up by the host macrophages when the sandfly feeds and differentiation into virulent amastigotes takes place.[3] Liposomal amphoteracin B is uptaken by macrophages and this is the only treatment currently approved by the US FDA[4] for use against visceral leishmaniasis.[5] Other methods that have been explored for selective drug delivery include liposomal entrapped antimony,[6,7] methotrexate-maleylated BSA conjugates,[8] pentamidine-loaded methactrylate nanoparticles,[9] aphidicolin nanosuspension formulations,[10] and 8-aminoquinoline analogs conjugated to *N*-acetylmannosamine containing polymers.[11] While these methods allow selective uptake by infected macrophages, little attention has been paid to enhancing a drug's affinity to the protozoa itself.

The lipophosphoglycan (LPG, Fig. 1),[12] the most abundant cell surface glycoconjugate on the promastigote, has been implicated in the invasion of macrophages by the protozoa and protects the organism from the hydrolytic environment of these immune cells.[13,14] The unique structure of the phosphodisaccharide repeating unit of the LPG displays an array of unsubstituted *cis*-vicinal diols and these offer an attractive target for reversible binding to boronic acids. Boronic acids are known to have a greater affinity for galactose and mannose than for glucose at physiological pH;[15] thus, drug molecules modified with boronic acids should selectively bind to the *Leishmania* cell surface. Oligomeric boronates should have an even higher affinity for the protozoan LPG if they are properly spaced to match the distance between each galactose and mannose in the repeating phosphodisaccharide. We have recently reported the synthesis of such oligomers based on δ-aminoboronic acids with varying length spacer units.[16] Here, we report modification of antileishmanial compounds using the same chemistry to further explore the potential method of using boronates in drug targeting.[17,18]

Pentamidine (**1**),[19] a drug that has long been used to treat leishmaniasis,[20] offers an excellent template on which to append boronic acids to bind to the LPG. In its extended form pentamidine spans over 18 Å from end to end—a distance greater than the maximal distance of separation between any two phosphates in the

Keywords: Boronate drug targeting; Pentamidine; *Leishmania*.
* Corresponding authors. Tel.: +61 7 3875 4115; fax: +61 7 3875 6572; e-mail: t.houston@griffith.edu.au
† Present address: Mayo Clinic, Rochester, MN, USA.

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.11.112

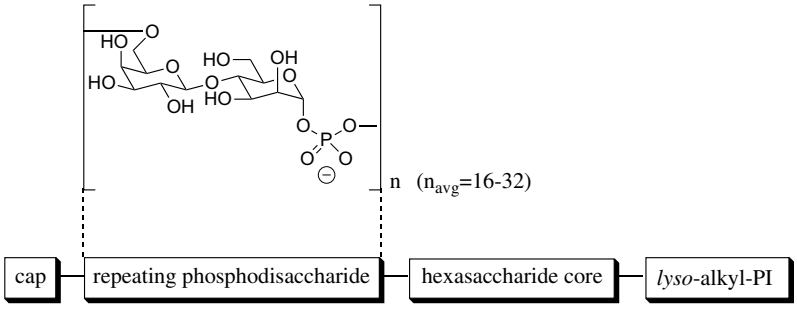


Figure 1. General structure of *Leishmania* LPG.[1]

repeat unit (<12 Å). Due to the dication formed in aqueous solution and the flexible nature of the pentyl linker in the drug, it should have some modest intrinsic affinity for the cell surface LPG. Addition of one or two boronic acids to both amidines would be expected to increase the compound's affinity for the LPG repeat. The reversible nature of boronate–carbohydrate binding would still allow the modified pentamidine to enter the cell while increasing the effective concentration of the drug at the cell surface. Pentamidine is a known DNA minor groove binder and can inhibit topoisomerase.[21] One question to be addressed by this work is whether the substitution of boronates on this core structure diminishes its effectiveness to kill the protozoa or whether it makes the drug more effective at lower concentrations by targeting it to the cell surface (Fig. 2).

To simplify the synthesis of boronic acid derivatives, a series of related diamines (**2**–**4**)[22] were targeted for study. The amidine group has been implicated in the toxicity of **1** through its affinity for the imidazoline I2 receptor and diamines based on **2** have been identified as novel DNA minor groove binding agents.[23] Other derivatives of the pentamidine core structure have antiprotozoal activity against *Leishmania* and related trypanosomes[24,25] and alternative topoisomerase inhibitors possess antileishmanial activity.[26] Original studies with pentamidine had shown that compounds with either a three or five carbon linker had the best trypanocidal activity.[19] Each of these offered sufficient spacing to allow both cationic ammonium species to bind to two phosphates in the LPG repeat unit simultaneously. Even the three-carbon linker of **5** allows for greater than 15 Å separation between the amines in extended form. The diamines **2**–**4**[22,23] and their boronic acid derivatives **5**–**7**[27] were readily synthesized through the following sequence: (1) reaction of 4-hydroxybenzonitrile with an appropriate dibromide, (2) nitrile reduction with LAH,[22] and (3) double reductive amination with *o*-formylphenylboronic acid.[16,28] Initially, mass spectral analysis of these compounds using FAB in either positive or negative ion modes gave molecular ion peaks much larger than expected. It became apparent that the diboronates were extracting two molecules of glycerol from the FAB matrix and this was confirmed by observing an increase in 32 mass units when rerunning the samples with a thioglycerol matrix. With these compounds in hand, their lethality to growing protozoa could then be tested in vitro to determine what effect

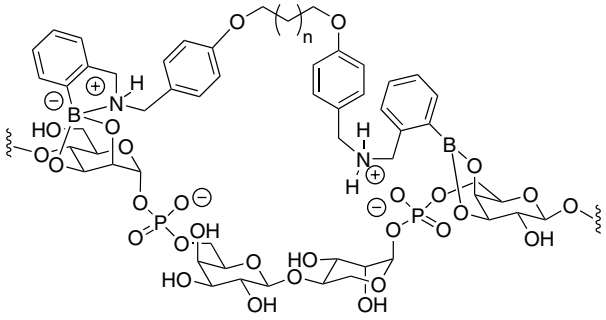
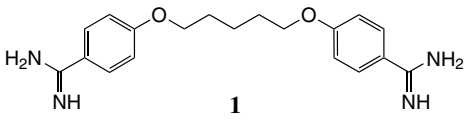
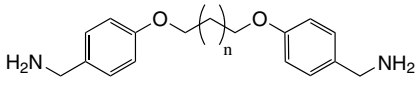


Figure 2. Possible binding modes of **5**–**7** to LPG.

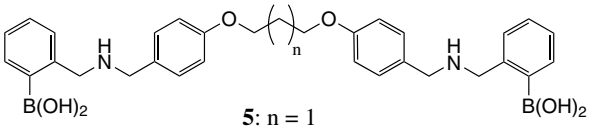
phenylboronate substitution had on the activity of the parent diamines.



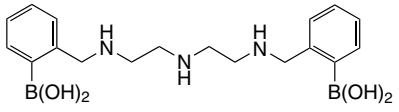
1



2: n = 1
3: n = 2
4: n = 3



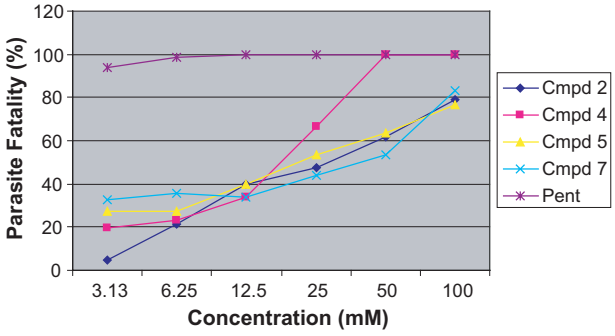
5: n = 1
6: n = 2
7: n = 3



8

As can be seen in Table 1,[29] the diamine derivatives are not as lethal as pentamidine to the protozoa (*Leishmania*

Table 1. Percent parasite fatality



chagasi promastigotes); however, some interesting trends are evident. The 4-carbon-linked compound **3** was less active (data not shown) than either **2** or **4**, which is consistent with the SAR of other pentamidine derivatives with the 5-carbon-linker (**4**) displaying best activity.[19] The boronate derivatives **5** and **7** of the 3-carbon-(**2**)[23] and 5-carbon-linked (**4**)[22] dibenzylic amines, displayed very similar activities indicating the boronate substitution does not drastically alter the desired biological activity of these compounds.

Importantly, the activities of the boronate derivatives appeared to decrease less rapidly than their respective diamines at lower concentration and this may be due to increased affinity for LPG that in turn increases its effective concentration around the organism. The results are consistent with targeted drug delivery where an affinity for the cell surface translates into increased uptake of drug by the protozoa (cf. even more dramatic is the difference between **2** and **5** at 3.13 mM). None of the dicyano precursors of **2**–**4** nor the diboronate **8**[16] displayed any cytotoxic effects against the protozoa at concentrations used here.

In conclusion, we have shown that the pentamidine derivative **4** and its diboronate analog **7** have similar potency versus *Leishmania* protozoa at low millimolar concentrations. While aromatic boronic acids are somewhat toxic, this adverse property should be reduced by the presence of an amine that can form an intramolecular Lewis acid–base complex. Not only would such a complex buffer the Lewis acidity of the boronate, but since it is charge neutral, it would also facilitate the compound's passage through biological membranes.[30–32] We are currently synthesizing more complex boronate derivatives in attempts to increase their potency and will study their binding properties to the repeat oligosaccharide of LPG.

Acknowledgements

We wish to thank Virginia Commonwealth University for support through the Mary Kapp Fund and Grant-In-Aid Program. Funding from the Jeffress Memorial Trust is also gratefully acknowledged. We also thank Professor Richard D. Pearson (U. Virginia) for supplying the *Leishmania* protozoa.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.11.112.

References and notes

1. Turco, S. J.; Descoteaux, A. *Annu. Rev. Microbiol.* **1992**, *46*, 65–94.
2. Sacks, D. L. *Exp. Parasitol.* **1989**, *69*, 100–103.
3. Sacks, D. L.; Modi, G.; Rowton, E.; Späth, G.; Epstein, L.; Turco, S. J.; Beverley, S. M. *PNAS* **2000**, *97*, 406–411.
4. Meyerhoff, A. *Clin. Infect. Dis.* **1999**, *28*, 42–48.
5. Cascio, A.; di Martino, L.; Occorsio, P.; Giacchino, R.; Catania, S.; Gigliotti, A. R.; Aiassa, C.; Iaria, C.; Giordano, S.; Colomba, C.; Polara, V. F.; Titone, L.; Gradoni, L.; Gramiccia, M.; Antinori, S. *J. Antimicrob. Chemother.* **2004**, *54*, 217–220.
6. New, R. R. C.; Chance, M. L.; Thomas, S. C.; Peters, W. *Nature (London)* **1978**, *272*, 55–57.
7. Tempone, A. G.; Perez, D.; Rath, S.; Vilharinho, A. L.; Mortara, R. A.; de Andrade, H. F. *J. Antimicrob. Chemother.* **2004**, *54*, 60–68.
8. Mukhopadhyay, A.; Chaudhuri, G.; Arora, S. K.; Sehgal, S.; Basu, S. K. *Science (Washington, DC)* **1989**, *244*, 705–707.
9. Durand, R.; Paul, M.; Rivollet, D.; Houin, R.; Astier, A.; Deniau, M. *Int. J. Parasitol.* **1997**, *27*, 1361–1367.
10. Kayser, O. *Int. J. Pharm.* **2000**, *196*, 253–256.
11. Nan, A.; Croft, S. L.; Yardley, V.; Ghandehari, H. *J. Controlled Release* **2004**, *94*, 115–127.
12. McConville, M. J.; Thomas-Oates, J. E.; Ferguson, M. A. J.; Homans, S. W. *J. Biol. Chem.* **1990**, *265*, 19611–19623.
13. Descoteaux, A.; Luo, Y.; Turco, S. J.; Beverley, S. M. *Science (Washington, DC)* **1995**, *269*, 1869–1872.
14. Späth, G. F.; Epstein, L.; Leader, B.; Singer, S. M.; Avila, H. A.; Turco, S. J.; Beverley, S. M. *PNAS* **2000**, *97*, 9258–9263.
15. Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291–5300.
16. Gray, C. W., Jr.; Walker, B. T.; Foley, R. A.; Houston, T. A. *Tetrahedron Lett.* **2003**, *44*, 3309–3312.
17. Johnson, L. L., Jr.; Houston, T. A. *Tetrahedron Lett.* **2002**, *43*, 8905–8908.
18. Yang, W.; Gao, S.; Gao, X.; Karnati, V. V. R.; Ni, W.; Wang, B.; Hooks, W. B.; Carson, J.; Weston, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2175–2177.
19. Ashley, J. N.; Barber, H. J.; Ewins, A. J.; Newbery, G.; Self, A. D. H. *J. Chem. Soc.* **1942**, 103–116.

20. Bray, P. G.; Barrett, M. P.; Ward, S. A.; de Koning, H. P. *Trends Parisitol.* **2003**, *19*, 232–239.
21. Henderson, D.; Hurley, L. H. *Nature Med.* **1995**, *1*, 525–527.
22. Burgess, L. E.; Newhouse, B. J.; Ibrahim, P.; Rizzi, J.; Kashem, M. A.; Hartman, A.; Brandhuber, B. J.; Wright, C. D.; Thomson, D. S.; Vigers, G. P. A.; Koch, K. *PNAS* **1999**, *96*, 8348–8352.
23. Johnson, H. A.; Thomas, N. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 237–241.
24. Donkor, I. O.; Huang, T. L.; Tao, B.; Rattendi, D.; Lane, S.; Vargas, M.; Goldberg, B.; Bacchi, C. *J. Med. Chem.* **2003**, *46*, 1041–1048.
25. Stephens, C. E.; Brun, R.; Salem, M. M.; Werbovetz, K. A.; Tanious, F.; Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2065–2069.
26. Slunt, K. M.; Grace, J. M.; MacDonald, T. L.; Pearson, R. D. *Antimicrob. Agents Chemother.* **1996**, *40*, 706–709.
27. Synthesis of diboronates **5**–**7**: The same general procedure was carried out throughout the series.[28] Compounds **2**[22,23] (225 mg, 0.78 mmol), **3**[22] (210 mg, 0.71 mmol), **4**[22] (200 mg, 0.64 mmol) were each dissolved in reagent grade MeOH (5 mL) along with *o*-formylphenylboronic acid (234 mg, 1.56 mmol; 212 mg, 1.42 mmol; 191.5 mg, 1.28 mmol; respectively) and these were allowed to stir at rt for 3–4 h. $NaBH_4$ (74 mg, 67 mg, 60.5 mg, respectively) was added and the reactions were stirred for an additional 2 h, then concentrated under vacuum. CH_2Cl_2 (10 mL) was added and the mixture was filtered. Dropwise addition of hexanes to the filtrate provided the products **5** (380 mg, 87% yield), **6** (200 mg, 48%), and **7** (225 mg, 62%) as white powders.
Compound **5**: ^{1}H NMR (300 MHz, CD_3OD) δ 2.24 (q, J = 6.2 Hz, 2H), 3.85 (s, 4H), 3.89 (s, 4H), 4.18 (t, J = 6.0 Hz, 4H), 7.00 (d, J = 8.8 Hz, 4H), 7.12 (m, 2H), 7.20 (m, 4H), 7.35 (d, J = 8.8 Hz, 4H), 7.50 (m, 2H) ppm. ^{13}C NMR (300 MHz, CD_3OD) δ 29.72, 51.03, 52.56, 64.42, 114.59, 126.35, 127.0, 128.0, 130.87, 141.5, 159.22 ppm. FAB-MS ($C_{37}H_{44}B_2N_2O_8$–H^+, diglycerol-boronate adduct) calcd 665.3_4, found 665.2_2.
Compound **6**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.97 (m, 4H), 3.86 (s, 4H), 3.92 (s, 4H), 4.10 (m, 4H), 6.98 (d, J = 8.8 Hz, 4H), 7.08 (m, 2H), 7.16 (m, 4H), 7.35 (d, J = 8.8 Hz, 4H), 7.45 (m, 4H) ppm. ^{13}C NMR (300 MHz, CD_3OD) δ 26.09, 50.49, 52.97, 67.60, 114.58, 126.43, 126.78, 127.02, 130.92, 141.3, 159.36 ppm. FAB-MS ($C_{38}H_{46}B_2N_2O_8$–H^+, diglycerol-boronate adduct) calcd 679.3_6, found 679.1_9.
Compound **7**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.70 (m, 2H), 1.85 (m, 4H), 3.85 (s, 4H), 3.90 (s, 4H), 4.00 (t, J = 6.2 Hz, 4H), 6.95 (d, J = 8.8 Hz, 4H), 7.05 (m, 2H), 7.15 (m, 4H), 7.35 (d, J = 8.8 Hz, 4H), 7.45 (m, 4H) ppm. ^{13}C NMR (300 MHz, CD_3OD) δ 22.77, 29.02, 50.48, 52.90, 67.80, 114.68, 126.56, 126.7, 127.15, 130.96, 141.2, 159.50 ppm. FAB-MS ($C_{39}H_{48}B_2N_2O_8$–H^+, diglycerol-boronate adduct) calcd 693.3_7, found 693.2_3.
28. Gray, C. W., Jr.; Houston, T. A. *J. Org. Chem.* **2002**, *67*, 5426–5428.
29. Compounds **2**–**7** were tested for leishmanicidal activity against the *L. chagasi* strain as follows. A 10 mM stock solution for each of the eight trials (six compounds in DMSO, pentamidine (**1**) in DMSO, and a DMSO control) were prepared. Each drug was diluted to a concentration of 400 μM with modified HOMEM buffer system. Addition of 40 μL DMSO in 460 μL HOMEM served as a solvent control. Initially, 100 μL of HOMEM was placed in all 12 rows of 8 columns of an 8 × 12 sterile cell well plate (FALCON 3872). Duplicate serial dilutions of each drug were made as follows: 100 μL of the 400 μM drug solution was pipetted into wells 1 and 2 (rows 1 and 2), 100 μL of the solution in well 1 was pipetted into well 3, and 100 μL of the solution in well 2 was pipetted into well 4. This procedure was repeated until 100 μL was removed from wells 11 and 12 at the end. To each well 100 μL of *L. chagasi* promastigotes at approximately 5 × 106 parasites/mL was added, and the cell well plate was placed in an incubator at 25 °C overnight. The parasites were counted after 24 and 48 h incubation time by pipetting 10 μL of solution from each well under the cover slip of a hemacytometer with a 5 × 5 major grid divided into 4 × 4 minor grids. The number of parasites in two major grids was counted (viewed at 400× magnification), averaged, and converted to parasites/mg by multiplying by 25 × 104. The average parasite counts were converted into percent parasite fatality as shown in Table 1.
30. Gardiner, S. J.; Smith, B. D.; Duggan, P. J.; Karpa, M. J.; Griffin, G. J. *Tetrahedron* **1999**, *55*, 2857–2864.
31. Draffin, S. P.; Duggan, P. J.; Duggan, S. A. M. *Org. Lett.* **2001**, *3*, 917–920.
32. Smith, B. D.; Davis, J. P.; Draffin, S. P.; Duggan, P. J. *Supramol. Chem.* **2004**, *16*, 87–90.