Bioorganic & Medicinal Chemistry Letters 21 (2011) 3890–3893

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Efficient synthesis, structural characterization and anti-microbial activity of chiral aryl boronate esters of 1,2-*O*-isopropylidene-α-D-xylofuranose

Rajiv Trivedi [a,*], E. Rami Reddy [a], Ch. Kiran Kumar [a], B. Sridhar [b], K. Pranay Kumar [c], M. Srinivasa Rao [c]

[a] Organometallic Group, I & P C Division, Discovery Lab D-214, Indian Institute of Chemical Technology, Hyderabad, Uppal Road, Tarnaka, Hyderabad 500007, India
[b] Center for X-ray Crystallography, Indian Institute of Chemical Technology, Hyderabad 500 007, India
[c] Chemical Biology Laboratory, Indian Institute of Chemical Technology, Hyderabad 500 007, India

ARTICLE INFO

Article history:
Received 14 March 2011
Revised 27 April 2011
Accepted 10 May 2011
Available online 18 May 2011

Keywords:
Chiral boronate esters
1,2-*O*-isopropylidene-α-D-xylofuranose
D-Xylose
Anti-bacterial activity
Anti-fungal activity

ABSTRACT

A simple and efficient synthetic approach toward a series of chiral aryl boronate esters, starting from D-xylose, as anti-microbial agents, is described herein. Minimum inhibitory concentration and zone of inhibition revealed that these derivatives exhibit potent anti-bacterial and anti-fungal properties. Herein, we report the first anti-microbial activity of this class of compounds. All products have been characterized by NMR (^{1}H, ^{13}C and ^{11}B), IR, elemental and mass spectral study.

Carbohydrates are ubiquitous in nature, readily available, cheap, biodegradable and non-toxic materials.[1,2] Presence of several functional groups and stereogenic centers in carbohydrates permit stereochemical differentiations, enantiopure compounds synthesis,[3–5] and their use as chiral templates,[6] biosensors[7] and as precursors for several biological active products.[8] Besides their crucial role, carbohydrates possess a unique set of chemical and structural features that make them particularly attractive as molecular scaffolds.

It is well documented that boron, as an essential trace element, has demonstrated significant relevance in a series of biochemical and biological processes such as protecting groups, enzyme inhibitors, affinity purification agents, receptors for saccharide sensing, neutron capturing agents for cancer therapy, bio-conjugates and protein lables.[9–17] Among the natural and synthetic boron compounds, several have displayed anti-bacterial, anti-fungal or anti-viral activities.[11–20] During the recent times, reports have described interesting activities of organoboron compounds such as: anti-HIV,[21] bacterial anti-inducer involved in quorum sensing,[22,23] and as therapeutics.[24,25]

Boronic esters of monosaccharides were first reported five decades ago.[26,27] The boronic esters of carbohydrates have essentially demonstrated their role as fluorescence sensors,[28–31] and as protecting groups.[26,27,32–35] Furthermore, it is well known that the diol functionalities of the carbohydrates form cyclic esters with boronic acids and make them more lipophilic and also increases transport through liquid organic membranes.[36] Klüfers and co-workers have tested the suitability of carbohydrate boronate esters as linear linkers for the formation of intrinsically chiral covalent organic frameworks (COF).[37] Nevertheless, taking into account the vast possibilities of carbohydrate structures incorporating boron, no reports on the use of carbohydrate based boronate esters in medicine is available.

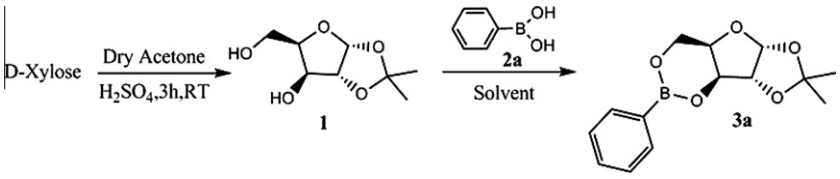
Based on our ongoing research interest in organoboron and carbohydrate chemistry, we intend to investigate the mutual interactions of boron and carbohydrates to produce a new class of anti-microbial agents. We hereby report a feasible and efficient synthesis, characterization and anti-microbial screening of carbohydrate based boronate esters. These compounds can be conveniently prepared in good yields and in a modular fashion, which leads to a better understanding of the structure–activity relationship. In the present study, we used gram-positive and gram-negative bacteria and fungi as representative microorganisms.

For the introduction of the boron moiety in carbohydrate frame work, we intended to use the known protected pentose sugar, 1,2-*O*-isopropylidene-α-D-xylofuranose **1**,[38] as the precursor because of its utility as a key intermediate in synthesis of antibiotics,[39] nucleosides,[40] herbicides,[41] anti-HIV agents,[42] and other biologically active compounds.[43] Thus, this compound was straightforwardly obtained from D-xylose as described by Moravcova et al.[38]

With protected diol **1** in hands, we turned our attention to the incorporation of the boron moiety in the furanoside back bone as shown in Scheme 1. A thorough literature search resulted in three reports for the synthesis of 1,2-*O*-isopropylidene-α-D-xylofuranose-3,5-phenylboronate **3a**.[44–46] However, these methods suffer

* Corresponding author. Tel.: +91 40 2719 1667; fax: +91 40 2716 0921.
E-mail address: trivedi@iict.res.in (R. Trivedi).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.05.036



Scheme 1. Synthesis of 1,2-*O*-isopropylidine-α-D-xylofuranose-3,5-phenyl boronate **3a** from D-xylose.

from various drawbacks such as use of toxic pyridine[45] as solvent, very low yields,[46] long reaction time and the removal of by products.[44] This motivated us to undertake the challenge to carryout ester formation reaction in a convenient way. The screening of solvents and reaction conditions were conducted for the formation of ester **3a** by the reaction of protected diol **1** (3 mmol) with phenylboronic acid **2a** (3 mmol). After screening the solvents and temperature parameters, we found that the best results were achieved by using Et_2O as solvent at room temperature. Under these conditions, diol **1** was cleanly converted into chiral aryl boronate ester **3a** and the product was isolated in 92% yield. Experiments conducted using toluene,[47] THF and EtOAc did not result in good yields of the desired product. The boronic esters were synthesised using the optimized condition[48] and different aryl boronic acids **2b–2j** as starting materials, as shown in Table 1. Electron donating and electron withdrawing groups attached at the *para* position were efficiently used to prepare boronate esters **3b–3c** and **3e–3g** in the range of 87–97% yield (Table 1, entries b–c and e–g). Aryl boronic acid carrying electron withdrawing substituent at the *meta* position was also prepared in good yield 95% (Table 1, entry h). 3-Thiophenyl, 2-thiophenyl and 2-naphthyl boronate esters **3d**, **3i** and **3j**, respectively, were also prepared in the range of 75–83% yield (Table 1, entries d, i and j). All the products described in the Table 1 were characterized by ^1H, ^{13}C, ^{11}B NMR, IR, elemental analysis and EI-MS (see Supplementary Data).

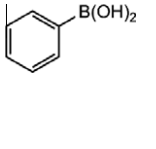
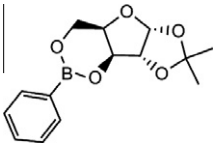
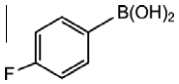
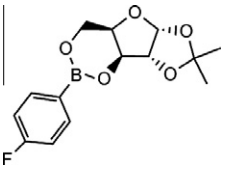
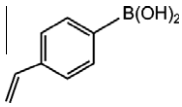
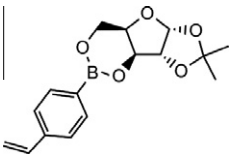
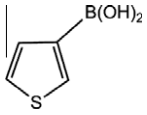
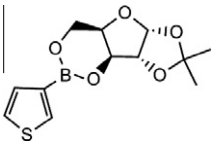
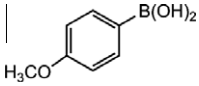
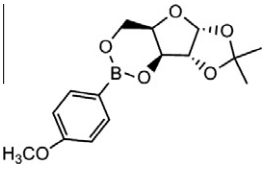
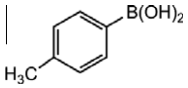
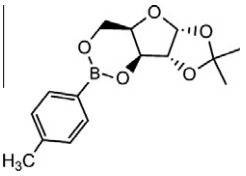
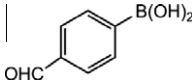
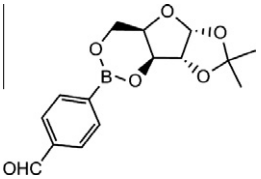
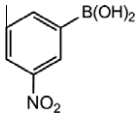
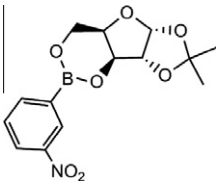
The IR spectra of all the cyclic boronate derivatives **3a–3j** exhibited three characteristic bands B–O, B–C and C–O absorption in the regions 1380–1305 cm^{-1}, 1081–1012 cm^{-1} and 1145–1167 cm^{-1}, respectively. The other bands were well matched with the other characteristic functional groups.

^1H NMR and ^{13}C NMR values were well assigned to all boronate esters.[49] The NMR signals of the aromatic *ipso* carbon atoms were not detectable may be due to fast relaxation caused by the magnetic interaction with the boron nuclei.[37] Thus, the ^1H NMR and ^{13}C NMR spectra did not yield a final proof for the existence of a cyclic system, such as a six-membered ring.[47] The ^{11}B NMR spectral data shows only one broad singlet peak in the region of δ 24.6–26.9 ppm, discloses that the boron atom remains three coordinate.[19,49,50] All the six-membered cyclic boronates **3a–3j**, showed the characteristic fragmentation structures[51] in mass spectral study (see Supplementary Data).

To obtain the precise three-dimensional structural information, the compound **3a** was characterized by single crystal X-ray diffraction studies, which confirms the six-membered boronate ring existence.[37,49,50] ORTEP diagram of **3a** was shown in Figure 1. The α-furanose ring adopts an envelope conformation on C4 being the extraplanar atom (puckering parameters[52]: Q2 = 0.362(2) Å and φ2 = 317.5(4)°). The crystallographic data[53] were listed in Supplementary Data.

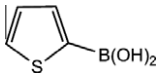
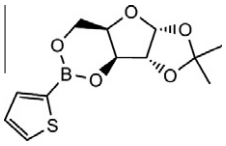
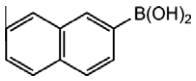
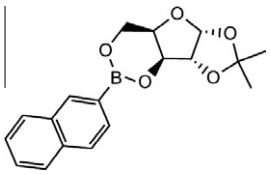
The minimum inhibitory concentrations (MIC) of various synthetic compounds were screened against three representative gram-positive microorganisms viz. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 2639) and gram-negative organisms viz. *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741) and *Klebsiella pneumoniae* (MTCC 618). The assays were performed by broth dilution

Table 1
Efficient synthesis of chiral aryl boronate esters **3a–3j**

Entry	Aryl bronicacid (**2**)	Product[a] (**3**)	Yield[b] (%)
a			92
b			90
c			93
d			78
e			87
f			88
g			97
h			95

(*continued on next page*)

Table 1 (*continued*)

Entry	Aryl bronicacid (**2**)	Product[a] (**3**)	Yield[b] (%)
i			75
j			83

[a] All compounds were characterized by NMR (^{1}H,^{13}C and ^{11}B), IR, elemental and mass spectroscopy.

[b] Yields refer to pure products after column chromatography.

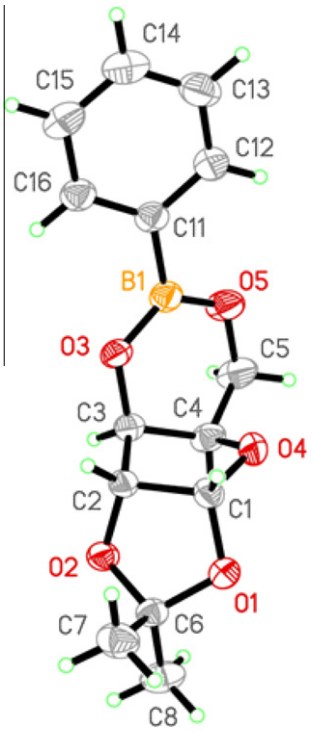


Figure 1. ORTEP diagram of compound **3a** (30% probability ellipsoids, hydrogen atoms with arbitrary radii).

techniques according to National Committee for Clinical Laboratory (NCCL) standards (1).[54] Standard anti-bacterial agents like Penicillin and Streptomycin were also screened under identical conditions for comparison. The minimum inhibitory concentration (MIC) values are presented in Table 2.

These results clearly indicate that compounds **3a–3j** displayed significant activity with a high degree of variation. The results also demonstrated that the activity of these compounds is influenced by their structures, the most effective (lower MIC values) being boronate esters **3g** and **3h** (Table 2). Moderate activity was shown by the compounds **3a**, **3b** and **3d** against all the tested organisms. Compound **3c** showed some activity against *P. aeruginosa*. Compound **3i** is active against *S. epidermidis* to some extent. The compounds **3e**, **3f** and **3j** have not shown any appreciable activity compared to other compounds.

In vitro anti-fungal activity of the synthesized boronate esters was studied against the fungal strains, *Candida albicans* (MTCC 227), *Candida rugosa* (NCIM 3462), *Saccharomyces cerevisiae* (MTCC 36) and *Aspergillus niger* (MTCC 282) by Agar Well Diffusion Method (2). The ready-made Potato Dextrose Agar (PDA) medium (Himedia, 39 g) was suspended in distilled water (1000 ml) and heated to boiling until it dissolved completely, the medium and Petri dishes were autoclaved at pressure of 15 lb/inc[55] for 20 min. Agar well bioassay was employed for testing anti-fungal activity and the results were shown in Table 3.

The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates solidified, 0.5 ml of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in DMSO and different concentrations were made. After inoculation, wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of test solutions were added. Controls were maintained. The treated and the controls were kept at 27 °C for 48 h. Inhibition zones were measured and the diameter was calculated in millimetre (mm). Three to four replicates were maintained for each treatment.

The anti-fungal screening data of compounds **3a**–**3j** revealed that all the tested compounds showed moderate to good anti-fungal activities against the tested fungal strains. Standard anti-fungal drug, amphotericin-B was also screened under identical conditions for comparison. Zone of inhibition (in mm) values indicates that the compounds **3g** and **3h** (Table 3) showed better anti-fungal activity against all fungal strains. The compounds **3b** and **3j** showed appreciable activity against all the tested fungal strains except *A. niger*. The compound **3a** showed only active against *A. niger*. The compound **3c** displayed activity except *C. rugosa*. The compound **3d** showed better activity against all strains except

Table 2
Anti-bacterial activity of compounds **3a–3j**

	MIC (μg/ml)					
Compd code	*B. Subtilis*	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
3a	37.5	75	18.75	18.75	37.5	75
3b	75	18.75	75	37.5	75	37.5
3c	75	75	75	150	37.5	150
3d	75	37.5	18.75	4.68	18.75	75
3e	150	150	150	150	150	75
3f	150	150	150	150	150	150
3g	18.75	9.375	18.75	4.68	37.5	4.68
3h	2.34	4.68	9.375	9.375	18.75	4.68
3i	75	150	37.5	150	75	150
3j	150	150	75	150	150	150
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

Table 3
Anti-fungal activity of compounds **3a–3j**

Compd code	Zone of Inhibition (mm)							
	C.albicans		*C.rugosa*		*S.cerevisiae*		*A. niger*	
	100 μg	150 μg	100 μg	150 μg	100 μg	150 μg	100 μg	150 μg
3a	0	6	0	0	0	0	8	12
3b	6	8	11	14	7	10	0	6
3c	6	9	0	0	6	8	9	14
3d	0	7	14	20	10	14	12	16
3e	0	0	7	10	0	0	0	0
3f	0	0	0	6	0	0	0	0
3g	12	14	13	17	14	19	6	8
3h	13	18	16	20	11	16	14	18
3i	0	6	9	13	12	17	9	11
3j	6	9	13	16	7	11	0	4
Amphotericin B	23.5		21		22		25	

C. albicans. The compound **3e** displayed some anti-fungal activity against *C. rugosa* only. The compound **3f** showing no activity against the tested organisms. The compound **3i** displayed some anti-fungal against all the tested strains except *C. albicans*.

The above result (Tables 2 and 3) discloses that some of the synthesized boronate esters are active against tested bacteria and fungi. Among all the boronate esters, **3g** and **3h** displayed better anti-bacterial and anti-fungal activities against all tested microorganisms.

In summary, the present work describes the preparation of chiral aryl boronate esters of 1,2-*O*-isopropylidene-α-D-xylofuranose. This synthetic approach is easy and efficient to prepare a wide range of boronate esters in a single step sequence at room temperature. These compounds are found to possess interesting antimicrobial activity. The minimum inhibitory concentration and zone of inhibition revealed that the activity of these compounds is modulated by structural modifications in the aryl group attached to boron atom.

Acknowledgements

This work was financially supported by the project fund DST Fast-TrackProject, GAP-0131. E.R.R and Ch.K.K are grateful to CSIR, New Delhi for the award of a fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.036.

References and notes

1. Holmberg, K. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 148.
2. Stubenrauch, C. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 160.
3. Hollingsworth, R. I.; Wang, G. *Chem. Rev.* **2000**, *100*, 4267.
4. Iglesias-Guerra, F.; Romero, I.; Alcudia, F.; Vega-Perez, J. M. *Carbohydr. Res.* **1998**, *308*, 57.
5. Iglesias-Guerra, F.; Candela, J. I.; Bautista, J.; Alcudia, F.; Vega-Perez, J. M. *Carbohydr. Res.* **1999**, *316*, 71.
6. Kunz, H.; Ruck, K. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 336.
7. Jelinek, R.; Kolusheva, S. *Chem. Rev.* **2004**, *104*, 5987.
8. Tatsuta, K.; Hosokawa, S. H. *Chem. Rev.* **2005**, *105*, 4707.
9. Dembitsky, V. M.; Al-Quntar, A. A. A.; Srebnik, M. *Chem. Rev.* **2011**, *111*, 209.
10. Baker, S. J.; Tomsho, J. W.; Benkovic, S. *J. Chem. Soc. Rev.* **2011**. doi:10.1039/c0cs00131g.
11. Huetter, R.; Keller-Schierlein, W.; Knuesel, F.; Prelog, V.; Rodgers, G. C., Jr.; Suter, P.; Vogel, G.; Voser, W.; Zaehner, H. *Helv. Chim. Acta* **1967**, *50*, 1533.
12. Dembitsky, V. M.; Smoum, R.; Al-Quntar, A. A.; Abu Ali, H.; Pergament, I.; Srebnik, M. *Plant. Sci.* **2002**, *163*, 931.
13. Bolanos, L.; Lukaszewski, K.; Bonilla, I.; Blevins, D. *Plant Physiol. Bio Chem. (Amsterdam)* **2004**, *42*, 907.
14. Hall, D. G. *Boronic Acids*, first ed.; Wiley-VCH: Weinheim, 2005. pp 1–549.
15. Berger, M.; Mulzer, J. *J. Am. Chem. Soc.* **1999**, *121*, 8393.
16. Matteson, D. S. *Med. Res. Rev.* **2008**, *28*, 233.
17. Abu Ali, V.M. Dembitsky, M. Srebnik (Eds.), In *Contemporary Aspects of Boron Chemistry and Biological Applications*, Elsevier: Amsterdam, 2005.
18. Eidam, O.; Romagnoli, C.; Caselli, E.; Babouglu, K.; Pohlhaust, D. T.; Karpiak, J.; Bonnet, R.; Shoichet, B. K.; Prati, F. *J. Med. Chem.* **2010**, *53*, 7852.
19. Duguay, D. R.; Zamora, M. T.; Blacquiere, J. M.; Appoh, F. E.; Vogels, C. M.; Wheaton, S. L.; Baerlocher, F. J.; Decken, A.; Westcott, S. A. *Cent. Eur. J. Chem.* **2008**, *6*, 562.
20. Zhu, Y.; Zhao, X.; Zhu, X.; Wu, G.; Ma, Y.; Yuan, Y.; Yang, J.; Hu, Y.; Ai, L.; Gao, Q. *J. Med. Chem.* **2009**, *52*, 4192.
21. Kohno, J.; Kawahata, T.; Otaka, T.; Morimoto, M.; Mori, H.; Ueba, N.; Nishio, M.; Kinumaki, A.; Komatsubara, S.; Kawashima, K. *Biosci., Biotechnol., BioChem.* **1996**, *60*, 1036.
22. Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. *Nature (London)* **2002**, *415*, 545.
23. Hodgkinson, J. T.; Welch, M.; Spring, D. R. *Acs. Chem. Biol.* **2007**, *2*, 715.
24. Adams, J.; Behnke, M.; Chen, S. W.; Cruickshank, A. A.; Dick, L. R.; Grenier, L.; Klunder, J. M.; Ma, Y. T.; Plamondon, L.; Stein, R. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333.
25. Priestley, E. S.; De, L. I.; Ghavimi, B.; ErickSon-Viitanen, S.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3199.
26. Kuivila, H. G.; Keough, A. H.; Soboczenski, E. J. *J. Org. Chem.* **1954**, *19*, 780.
27. Sugihara, J. M.; Bowman, C. M. *J. Am. Chem. Soc.* **1958**, *80*, 2443.
28. James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159.
29. Arimori, S.; Bell, M. L.; Oh, C. S.; Frimat, K. A.; James, T. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 803.
30. Cooper, G. J. T.; Cronin, L. *Nat. Chem.* **2009**, *1*, 342.
31. Gardner, P. M.; Winzer, K.; Davis, B. G. *Nat. Chem.* **2009**, *1*, 377.
32. Ferrier, R. J. *Adv. Carbohydr. Chem. Biochem.* **1978**, *35*, 31.
33. Mikami, M.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1995**, 153.
34. Dahlhoff, W. V.; Koster, R. *Synthesis* **1980**, 936.
35. Dahlhoff, W. V.; Fenzl, W.; Koster, R. *Liebigs Ann. Chem.* **1990**, 807.
36. Bien, J. T.; Shang, M.; Smith, B. D. *J. Org. Chem.* **1995**, *60*, 2147.
37. Reichvilser, M. M.; Heinzl, C.; Klufers, P. *Carbohydr. Res.* **2010**, *345*, 498.
38. Moravcova, J.; Capkova, J.; Stanek, J. *Carbohydr. Res.* **1994**, *263*, 61.
39. Matsuda, F.; Terashima, S. *Tetrahedron* **1988**, *44*, 4721.
40. Gosselin, G.; Bergogne, M. C.; Imbach, J. L. *Nucleic Acid Chem.* **1991**, *4*, 41.
41. Loh, W. U.S. Pat. 4,515,618, 1985.
42. Almond, M. R.; Wilson, J. D.; Rideout, J. L. U.S. Pat. 4,916,218, 1990.
43. Achab, S.; Das, B. C. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2863.
44. Ferrier, R. J.; Prasad, D.; Rudowski, A. *J. Chem. Soc.* **1965**, 858.
45. Wood, P. J.; Siddiqui, I. R. *Carbohydr. Res.* **1974**, *32*, 97.
46. Stacey, B. E.; Tierny, B. *Carbohydr. Res.* **1976**, *49*, 129.
47. Meiland, M.; Heinze, T.; Guenther, W.; Liebert, T. *Tetrahedron Lett.* **2009**, *50*, 469.
48. *General procedure for the synthesis of chiral aryl boronate ester*(**3a–3j**): A mixture of selected aryl boronic acid **2** (3 mmol) and 1,2-*O*-isopropylidene-α-D-xylofuranose **1** (3 mmol) were dissolved in anhydrous Et_2O; the mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% EtOAc/hexane) to afford a white crystalline solid.
49. Smoum, R.; Rubinstein, A.; Srebnik, M. *Magn. Reson. Chem.* **2003**, *41*, 1015.
50. Ketuly, K. A.; Hadi, A. H. A. *Molecules* **2010**, *15*, 2347.
51. Reinhold, V. N.; Wirtz-Peitz, F.; Biemann, K. *Carbohydr. Res.* **1974**, *37*, 203.
52. Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354.
53. Crystallographic data for the structure of **3a** in this Letter has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 814939 .Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax:+44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
54. National Committee for Clinical Laboratory (NCCL). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, which Grows Aerobically*, 5th ed.; Approved Standard M7-A5, NCCLS: Villanova, PA, 2000.
55. Linday, M. E. *Practical Introduction to Microbiology*; E and F. N. Spon ltd: United Kingdom, 1962.