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Journal of Organometallic Chemistry 493 (1995) 91–94

Journal of Organometallic Chemistry

Synthesis and structural investigation of two potential boronate affinity chromatography ligands catechol [2-(diisopropylamino)carbonyl]phenylboronate and catechol [2-(diethylamino)carbonyl, 4-methyl]phenylboronate

Xiao-Chuan Liu *, John L. Hubbard, William H. Scouten

Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, USA

Received 14 September 1994; in revised form 7 November 1994

Abstract

Two potential boronate affinity chromatography ligands, catechol [2-(diisopropylamino)carbonyl]phenylboronate (**I**) and catechol [2-(diethylamino)carbonyl,4-methyl]phenylboronate (**II**) were synthesized by directed ortholithiation followed by boronation. Single crystal X-ray analyses of compounds **I** and **II** demonstrated an internal coordination bond between the boron atom and the carbonyl oxygen atom, rendering the boron atom environment to be tetrahedral. In addition, ^{11}B NMR data also indicated that the boron environment is tetrahedral. The coordinated carbonyl oxygen–B bond length is 1.556(9) Å compared to an average B–O bond length of 1.47 Å to the catechol ligand. They are ideal models of a new type of ligands to study boronate affinity chromatography because they may esterify with catechols at neutral pH conditions.

Keywords: Boron; Boronate; Boronic acid; Crystal structure; Chromatography

1. Introduction

The use of boronate affinity chromatography for separation of nucleic acid components and carbohydrates was first reported by Weith et al. in 1970 [1]. Since then, the specificity of boronate groups has been exploited for the separation of *cis*-diol containing compounds, including catechols, nucleic acids, glycoproteins and carbohydrates [2]. A few recent examples of such applications can be found in Refs. [3–14]. The earliest and most widely used ligand for boronate chromatography is 3-aminophenylboronate acid (3aPBA). In chromatography using 3aPBA, the pH must be basic, i.e. pH > 8. The pKa of 3aPBA is 8.8, so that for optimum binding, the pH should be as high as possible for use with proteins and similar biomolecules. However, in many cases biomolecules lose their biological activity at pH values much above pH 7.5. This limits the use of this ligand in boronate affinity chromatography. A number of efforts have been made by researchers to lower the pKa of boronate ligands. In earlier studies, ligands such as *p*-bromophenylboronate [15], *p*-(w-aminoethyl)phenylboronate [16], and *p*-vinylbenzeneboronate [17] were employed. Recently, several investigatiors tried to introduce a strong electron withdrawing group on benzene ring to lower the pKa of ligands. For example, Hageman et al. put a (*N*-methyl)carboxyamido- group on a boronate containing benzene ring [18]; while Singhal et al. used a nitro substituted benzene boronate [19]. In spite of these efforts, the goal of obtaining a ligand which can form a complex with *cis*-diol compounds at neutral pH has not been attained [20].

Increasing evidence shows that a tetrahedral boronate is the favorable configuration for exchanging hydroxyls with *cis*-diol compounds [21], since the tetrahedral form is the active form [22]. This may be because the tetrahedral boronate has a more stable and less strained configuration than the trigonal form. In order to better understand the structure of these boronates, we synthesized compounds **I** and **II** and investigated their structures using X-ray crystallography and NMR spectroscopies.

* Corresponding author.

SSDI 0022-328X(94)05337-5

2. Experimental details

2.1. Synthesis of catechol [2-(diisopropylamino)carbonyl]phenylboronate (I) and catechol [2-(diethylamino)carbonyl, 4-methyl]phenylboronate (II)

A modification of the method described by Beak and Brown [23] was used to synthesize I and II (Fig. 1). All glassware and syringes were oven dried. The reactions were carried out under prepurified nitrogen. THF was freshly distilled from potassium metal under nitrogen. TMEDA was freshly distilled from calcium hydride under nitrogen. Secondary butyl lithium was standardized prior to use by titration using 2,5-dimethoxybenzyl alcohol as an indicator [24]. A detailed procedure for the synthesis of catechol [2-(diisopropylamino) carbonyl]-phenylboronate (I) is given below.

To 2 ml of TMEDA (13.0 mmol) in 30 ml dry THF at −78 °C was added dropwise 12 ml ^{sec}BuLi (13.0 mmol). After 10 min, 2.3 g of *N,N*-diisopropyl benzamide (11.0 mmol) in 20 ml dry THF was added dropwise. The mixture was then stirred for 1 h at −78 °C. Trimethyl borate 8.0 ml (70.4 mmol) was added rapidly and the mixture was then stirred for 20 h during which time it was allowed to warm to room temperature. The reaction mixture was then poured into a separating funnel containing saturated aqueous NH_4Cl (PH 5.7) and ether. The water phase was extracted twice and the combined ether phase was dried over $MgSO_4$. Evaporation of solvent in vacuo gave 2.23 g (9.0 mmol, 81.5%) [2-(diisopropylamino)carbonyl]phenylboronic acid as a colorless syrupy liquid.

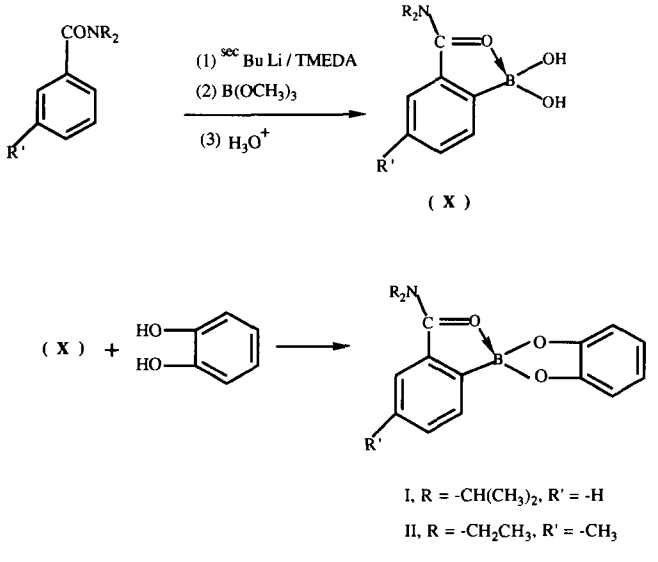

Fig. 1. Synthesis of two potential boronate affinity chromatography ligands catechol [2-(diisopropylamino)carbonyl]phenylboronate (I) and catechol [2-(diethylamino)carbonyl, 4-methyl]phenylboronate (II).

Using a Dean-Stark water separator, the azeotrope of water and benzene was distilled from a solution of 2.2 g (8.8 mmol) of [2-(diisopropylamino)carbonyl]phenylboronic acid in 100 ml benzene. The reaction mixture cooled to 70 °C and 1.0 g of catechol (9.1 mmol) was added. Distillation was then continued for 3 h. Evaporation of the solvent in vacuo yielded 2.41 g (84.8%) of crude product. Recrystallization from toluene gave white needles of compound I that contains one molecule of catechol of crystallization. m.p. 179–180 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 7.78 (d, 1H), 7.68 (d, 1H), 7.60 (t, 1H), 7.42 (t, 1H), 6.80 (m, 4H), 5.05 (m, 1H), 3.80 (m, 1H), 1.46 (q, 12H). ^{11}B NMR δ(ppm) 13.5 ppm, single peak. IR (KBr), ν 1620 cm^{-1}, 746 cm^{-1}. Mass spectrum (*m/z*, ion): 323, 280, 223, 195, 167, 136, 105. HRMS, observed: 323.1699; accurate: 323.1692. Anal. Calc. for $C_{19}H_{22}O_3BN \cdot C_6H_6O_2$: C, 69.30; H, 6.51; N, 3.23. Found: C, 69.21; H, 6.57; N 3.13.

Catechol [2-(diethylamino)carbonyl,4-methyl]phenylboronate (II) was synthesized according to the same procedure, using *N,N*-diethyl-m-tolumide in place of *N,N*-diisopropyl benzamide. m.p. 173–175 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) 7.66 (d, 1H), 7.50 (s, 1H), 7.44 (d, 1H), 6.88 (m, 2H), 6.78 (m, 2H), 3.95 (q, 2H), 3.70 (q, 2H), 2.45 (s, 3H), 1.50 (t, 3H), 1.30 (t, 3H). ^{11}B NMR δ(ppm) 13.5 ppm, single peak. IR (KBr), ν 1620 cm^{-1}, 806 cm^{-1}, 740 cm^{-1}, 701 cm^{-1}. Mass spectrum (*m/z*, ion): 295, 223, 195, 167 105. HRMS, observed: 295.1385; accurate: 295.1380. Anal. Calc. for $C_{18}H_{20}O_3BN \cdot 0.5C_7H_8$: C, 72.69; H, 6.81; N, 3.94. Found: C, 72.16; H, 6.68; N, 3.91.

2.2. X-ray crystallography

A colorless needle of suitable size was selected from a solution of the compound (I) that had been concentrated by slow evaporation. The crystal was coated in silicone grease and mounted inside a 0.3 mm X-ray capillary. After centering optically in the beam (1 mm dia) of a Siemens P4 diffractometer, the centering of 25 randomly chosen reflections with $15° \leqslant 2\theta \leqslant 30°$ revealed a primitive monoclinic Bravias lattice. The intensities of two check reflections measured every 50 reflections were constant within 2% throughout the data collection. The space group $P2_1$ was chosen on the basis of systematic absences and the successful solution by direct methods. Solution by direct methods in the centrosymmetric space group $P2_1/m$ was not successful. In addition, the $E^2 - 1$ value of 0.767 for the data set was significantly lower than the 0.968 value expected for the centrosymmetric space group. Full matrix least-squares refinement was performed to convergence with all nonhydrogen atoms anisotropic and the H-atoms generated in idealized locations with fixed (0.08) thermal parameters. All solution and refinement procedures

Table 1
Selected bond lengths (A) and bond angles (°) for (I)

B–O(1)	1.466(10)	B–O(2)	1.474(8)
B–O(3)	1.556(9)	B–C(7)	1.587(10)
O(1)–B–O(2)	105.0(5)	O(1)–B–O(3)	106.8(5)
O(2)–B–O(3)	107.9(6)	O(1)–B–C(7)	118.5(7)
O(2)–C–C(7)	118.1(6)	O(3)–B–C(7)	99.5(5)

utilized the SHELXTL-PLUS package of programs available from Siemens (Siemens Corp., Madison, WI).

Crystal data for compound I ($C_{25}H_{28}NO_5B$): Monoclinic space group $P2_1$, $a = 10.357$ (5) Å, $b = 9.149$ (3) Å, $c = 13.428$ (6) Å; $\beta = 110.21$ (4)°, $V = 1194.0$ Å^3, $Z = 2$, d(calc) = 1.21 g cm^{-3}, $\lambda = 0.71073$ Å, $R/R_w = 0.0605/0.0614$ for 1310 independent reflections ($F > 3\sigma$), goodness-of-fit = 1.19. Selected bond distances and bond angles are listed in Table 1. Complete details of data collection and refinement are deposited with Cambridge Crystallographic Data Center.

The compound II was recrystallized from toluene, monoclinic space group Cc, $a = 17.531(7)$, $b = 9.549(3)$, $c = 22.752(7)$, $\beta = 93.65(3)°$, $V = 3801.0(4)$ Å, $F(000) = 1920$, $\mu = 0.12$ mm^{-1} data collection at −100 °C. The structure that resulted from direct methods was essentially the same as compound I but the weakness of the data prevented the R value from dropping below 15%. Low resolution structure features for compound II are available from the authors upon request.

3. Results and discussion

Directed orthometalation is a useful method for synthesis of a variety of substituted aromatics [25]. In our study, directed ortholithiation followed by boronation is used and found to be a simple way to synthesize arylboronic acids. Because boronic acids are difficult to crystallize, there are very few crystal structures reported, e.g. phenylboronic acid [26] and *o*-formylbenzeneboronic acid [27]. There were only 13 boronic ester crystal structures listed in the Cambridge Structure Database. Some representatives of related structures are six-membered ring esters of phenylboronic acid [28], derivatives of dibenzobicyclic phenylboronate [29] and 4-ethyl-1-hydroxy-3-(4-hydroxyphenyl)-2-oxa-1-boranaphthalene [30].

The X-ray structure of compound I is presented in Fig. 2. The molecule of catechol that co-crystallized with I is not shown. Compound I shows an internal coordination bond between the carbonyl oxygen atom and the boron atom. ^{11}B NMR shows a single peak at 13.5 ppm, which is also an indication of a tetrahedral environment for the boron atom [31]. From Table 1 we see that the coordination around the boron atom is somewhat distorted from a regular tetrahedron. The bond angles around the boron are 99.5°, 105.0°, 106.8°, 107.9°, 118.1° and 118.5°, while in a normal tetrahedron such as in $(NCCH_2)(CF_3)_2B \cdot NH(CH_3)_2$, the bond angles around the boron are 107.9°, 108.0°, 110.8°, and 111.4°[32]. The average bond lengths between boron and the catechol oxygens are 1.47 Å, while the coordination bond length between boron and carbonyl oxygen of 1.56 Å is somewhat longer. This can be expected for a Lewis acid–base coordinate covalent bond. The plane defined by the [catechol-B] ring (mean standard deviation from planarity = 0.07 Å) lies at 93° relative to the plan defined by [$BO_3C_{13}C_{12}C_{11}C_{10}C_9C_8C_7$] (mean standard deviation from planarity = 0.05 Å). Nitrogen usually has a stronger ability to donate a pair of electrons for coordination than does oxygen, however the fact that the oxygen, rather than the nitrogen, in compound I donates a pair of electrons to the boron atom is probably due to the steric hindrance of two isopropyl groups associated with the nitrogen atom.

The only structure similar to compound I that has been previously reported is ethylene glycol [(1R)-1-acetamido-3-(methylthio)propyl]boronate [33], an ester of an aliphatic boronic acid. This compound also has an internal O–B coordination band. The bond angles around the boron in this case are 98.8°, 106.7°, 106.8°, 108.1°, 117.4°, and 118.0°, and the coordinated O–B length of 1.64 Å is longer than the covalent B–O length of 1.43 Å and 1.44 Å found in the ester linkages. Overall, the general features are very close to our data, being internally consistent and in agreement with other experimental values found in the Cambridge Structure Database [34].

In conclusion, our new boronate ligands are ideal models of a new type of ligands to study boronate affinity chromatography. Since the boron atom is in the

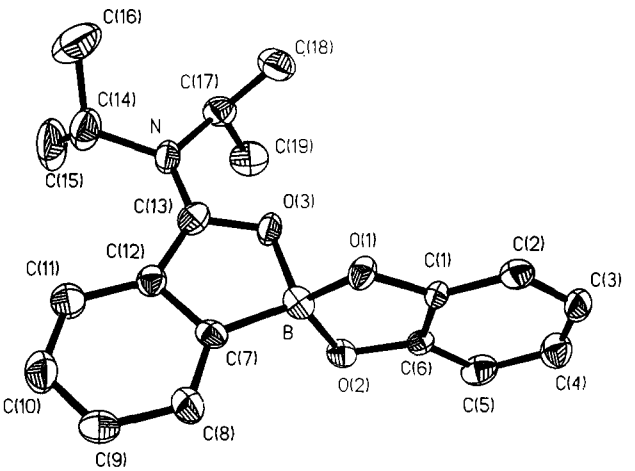

Fig. 2. X-ray crystal structure of a potential boronate affinity chromatography ligand, catechol [2-(diisopropylamino)carbonyl]phenylboronate (I).

active tetrahedral form, these ligands are expected to esterify with *cis*-diol compounds under neutral conditions, making them more valuable for biological applications. Futhermore, results from chromatography show they can esterify with catechol at neutral pH values, something that current commercial boronate affinity gels are unable to do [35].

Acknowledgments

We thank Dr. C. Garner at Baylor University for advice and discussion on the project; and Dr. V. Snieckus at University of Waterloo for suggestions on synthesis of arylboronic acid. X-ray diffractometer was funded by USU research office and matching funds from NSF (CHE-9002379). This research was supported by the Utah Agricultural Experiment Station, Utah State University, Logan, UT 84322-4810. Approved as journal paper no. 4679.

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