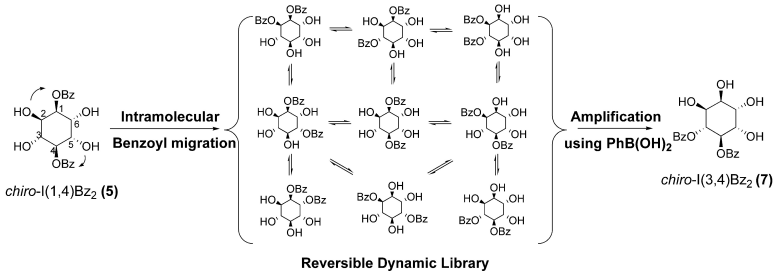
JOURNAL OF combinatorial CHEMISTRY

Report

Molecular Evolution on *chiro*-Inositol Dibenzoate Using Intramolecular Acyl Migration and Selection by Phenyl Boronic Acid

Young-Hoon Ahn, and Young-Tae Chang

J. Comb. Chem., **2004**, 6 (3), 293-296• DOI: 10.1021/cc030046z • Publication Date (Web): 15 April 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

JOURNAL OF combinatorial CHEMISTRY

Volume 6, Number 3 May/June 2004

Reports

Molecular Evolution on *chiro*-Inositol Dibenzoate Using Intramolecular Acyl Migration and Selection by Phenyl Boronic Acid

Young-Hoon Ahn and Young-Tae Chang*

Department of Chemistry, New York University, New York, New York 10003

Received November 14, 2003

Molecular evolution incorporating the dynamic combinatorial library (DCL) approach has identified new host−guest systems.[1] Two key features of molecular evolution are the selection and amplification of a particular species in the DCL. A DCL allows for a reversible equilibrium of library members in which a concentration change in one library member results in a new equilibrium distribution following Le Chatelier's principle. The molecular recognition event in the DCL induces the stabilization of the fittest binder, resulting in the shift of the equilibrium, amplifying the fittest binder with a decrease of others. To date, a number of DCLs have been prepared using such diverse chemical reactions as intermolecular transesterification,[2] enzyme catalyzed peptide-bond exchange,[3] imine bond exchange of hydrazones or oximes,[4] olefin metathesis,[5] disulfide bond exchange,[6] photoisomerization,[7] hydrogen bond exchange,[8] and metal−ligand coordination.[9] Although some DCLs have successfully demonstrated molecular evolution's proof of concept, the development of a highly efficient, practical system remained challenging. An ideal molecular evolution system requires an even distribution of the DCL components, an efficient selection method, and a nondestructive/continuous equilibrium in order to generate a "winning" binder for amplification. Herein, we report a highly efficient molecular evolution system utilizing intramolecular acyl migration on a carbohydrate scaffold coupled with boronic acid as a selector.

We have chosen hexahydroxyl cyclohexane (inositol) for our intramolecular acyl migration model system. In these molecules, each free hydroxyl group behaves as a nucleophile by attacking neighboring acyl groups, thus generating various regioisomers. Under basic conditions (pyridine/water), it was previously reported that benzoyl migration on *myo*-inositol (with five equatorial and one axial OH's) generates an almost equimolar amount of nine regioisomers.[10] To maximize the geometric diversity among regioisomers, we chose *chiro*-inositol (with two vicinal axial OH's and four equatorial OH) as DCL scaffold. Thus 1,4-dibenzoyl-*cihro*-inositol was synthesized[11] and investigated for optimization of the DCL generation. *Chiro*-inositol dibenzoate generates a total of nine regioisomers upon full equilibration (Scheme 1). A series of bases, including pyridine, DMAP, DABCO, DIEA, BEMP, DBU, and NaOH in aprotic solvents (DMF, DMSO, CH_3CN), all with and without water, have been evaluated. While several conditions induced benzoyl migration at a reasonable rate, generally speaking, increasing amounts of water not only accelerated the formation of the DCL (due to the increased polarity of solvent), but also increased the extent of hydrolysis of the benzoyl group. In addition, the migration rate strongly depends on the concentration and pK_b of the base and temperature. To minimize debenzoylation, we selected anhydrous acetonitrile as the solvent with DBU as a base. The optimal condition consisted of 1,4-dibenzoyl-*chiro*-inositol (**5**, 1 mg) and DBU (30 μL, 79 equiv) in acetonitrile (1 mL), yielding full migration within 1 h at room temperature. Generation of the nine isomers was confirmed by mass spectrometry and UV analysis using LC/MS (Figure 1a and b) (See the Supporting Information for mass spectrum).

Since it is well-known that boronic acids form five-membered cyclic esters, preferably with 1,2-*cis* diols, in sugar

* Corresponding author. Phone: (212)-998-8491. Fax: (212)-995-4203. E-mail: yt.chang@nyu.edu.

10.1021/cc030046z CCC: $27.50
Published on Web 04/15/2004

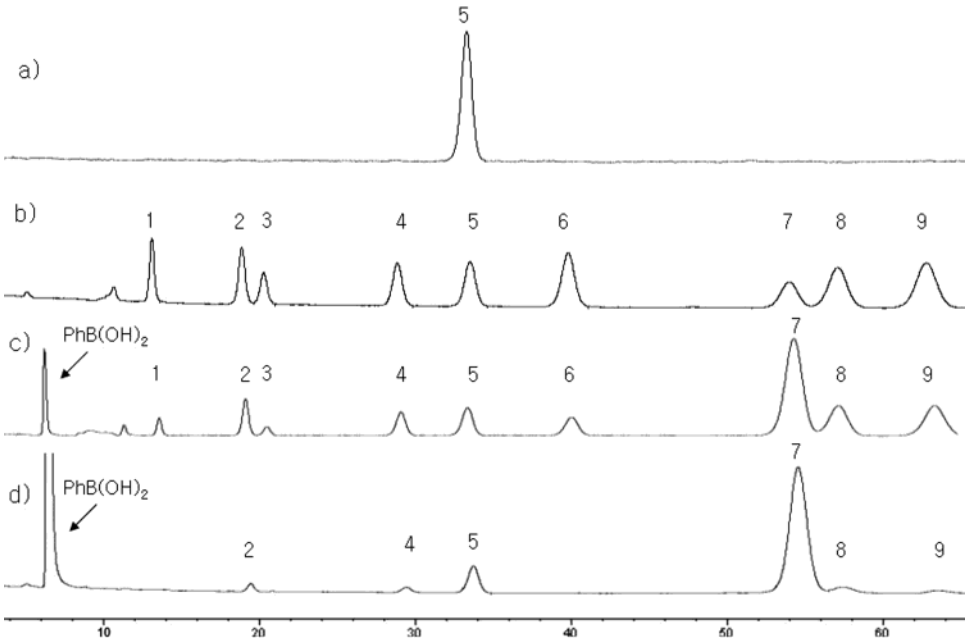
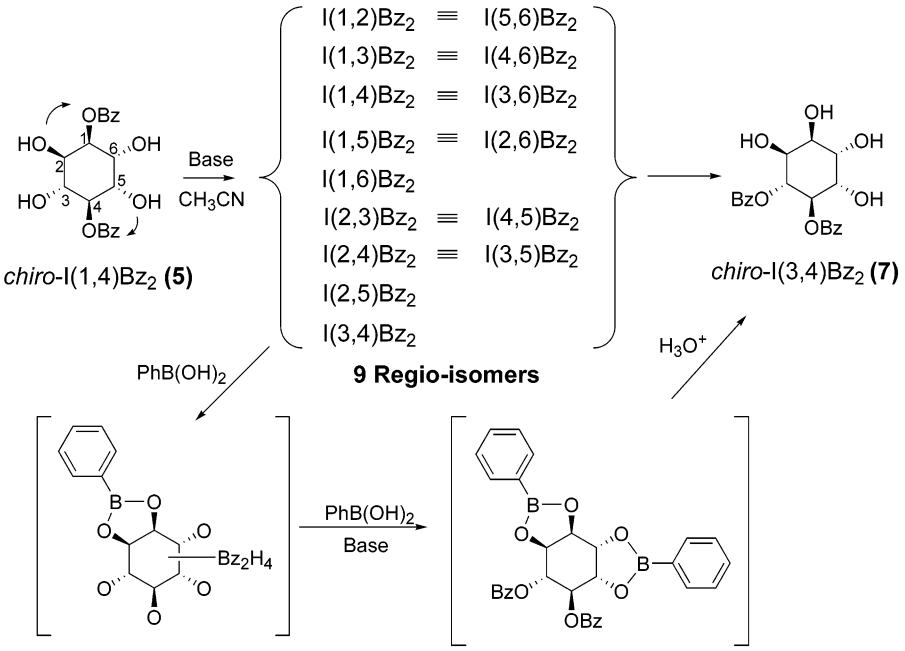


Figure 1. (a) HPLC chromatogram (C18 column: 4.6 × 150 mm, eluted with 18% acetonitrile in water) of *chiro*-I(1,4)Bz_2 (**5**) before base addition. (b) Chromatogram of the DCL at 1 h after addition of DBU with 8% of **7**. (c) Chromatogram of the DCL at 7 h after addition of $PhB(OH)_2$ (2 equiv) with 43% of **7**. (d) Chromatogram of the DCL at 7 h after addition of $PhB(OH)_2$ (32 equiv) with 82% of **7**. The percentage of each isomer was calculated on the basis of the integration in the HPLC trace at 250 nm.

Scheme 1. Library Members Generated by Acyl Migration and the Selected Component upon Treatment with Phenyl Boronic Acid



systems,[12,13] it was envisioned that only one isomer out of the nine, 3,4-dibenzoyl-*chiro*-inositol, which carries two cis-vicinal diols, would have a higher binding affinity for two boronic acids, thus stopping further migration. Furthermore, it was also reported that the binding affinity of boronic acid with a sugar depends on the pH of the media.[13] At pHs lower than the pK_a of boronic acid (acidic conditions), coupling between the boronic acid and a sugar is disfavored, whereas the coupling is favored at pHs over the pK_a of boronic acid (basic conditions).

To test the above hypothesis, a portion of phenyl boronic acid (32 equiv) was added to the equilibrium mixture of the *chiro*-inositol dibenzoates in the presence of DBU. A dramatic equilibrium shift of mixtures toward component **7**, characterized as 3,4-dibenzoyl-*chiro*-inositol by a separate synthesis,[11] was demonstrated (Figure 1d). Isomer **7**, initially 8% of the equilibrium mixture (Figure 1b) became the major constituent of the library (82%) in 7 h after treatment with DBU, demonstrating an enrichment factor of 10.3 (Figure 2).

It was envisioned that the nine regioisomers generated from compound **5** would initially couple with one phenyl boronic acid at the moment one *cis* diol in any component is exposed from benzoyl migration. Following that, the

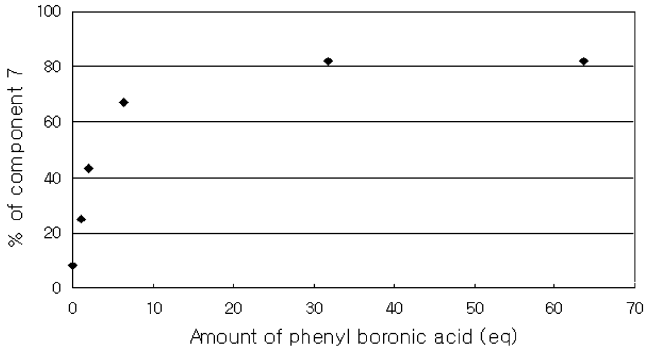


Figure 2. The amplification of **7** using various amounts of phenyl boronic acid at 7 h after preequilibrium. Reaction conditions: *chiro*-I(1,4)Bz$_2$ (**5**) (1 mg), DBU (30 μL, 79 equiv) in acetonitrile (1 mL) for 1 h, and then various amounts of phenyl boronic acid.

monoboronic acid-coupled component will undergo further acyl migration until a second *cis* diol is exposed and trapped by yet another phenyl boronic acid (Scheme 1). Benzoyl migration will terminate when two *cis* diols of 3,4-dibenzoyl *chiro*-inositol couple with two boronic acids, since free hydroxyl groups are no longer available. This effectively isolates this component from the equilibrating DCL, resulting in an accumulation of the "winning" binder. Thus, increased amounts of boronic acid will shift the equilibrium toward the selected compound, **7**. With 1, 2, 6, and 32 equiv of phenyl boronic acid, the final amount of **7** was 25, 43, 67, and 82%, respectively; however, amounts of phenyl boronic acid in excess of 32 equiv did not further amplify **7** (Figure 2).[14] The final distribution of the regioisomers is "quenched" by treating the reaction mixture with acetic acid that stops acyl migration. This "quenched" distribution allowed for a practical isolation of the product with the column chromatography purification, resulting in 72% recovery of **7** as a pure isomer.

We have demonstrated an efficient molecular evolution model by combining the base-catalyzed intramolecular acyl migration of inositol dibenzoate and a boronic acid selector. In the current example, we could amplify and accumulate up to 82% of the equilibrium mixture one component, **7**, out of a total of nine isomers originating from **5**. As a result, species **5** (Figure 1a) has been efficiently evolved into **7** (Figure 1d) with a boronic acid selector via nine mutants (Figure 1b). This is the first application of a boronic acid used to select and amplify a carbohydrate member of a DCL through intramolecular acyl migration. Further studies using various boronic acids on other sugar derivatives and with a more diverse library are under investigation in our laboratory.

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

Supporting Information Available. Experimental procedures and characterization data are available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Curr. Opin. Chem. Biol*. **2002**, *6*, 321−327. (b) Furlan, R. L. E.; Otto, S.; Sanders, J. K. M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4801−4804. (c) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898−952. (d) Ramström, O.; Bunyapaiboonsri, T.; Lohmann, S.; Lehn, J. M. *Biochim. Biophys. Acta* **2002**, *1572*, 178−186. (e) Lehn, J. M.; Eliseev, A. V. *Science* **2001**, *291*, 2331−2332. (f) Karan, C.; Miller, B. L. *Drug Discovery Today* **2000**, *5*, 67−75. (g) Ganesan, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2828−2831.

(2) (a) Brady, P. A.; Bonar-Law, R. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K. M. *Chem. Commun.* **1996**, 319−320. (b) Rowan, S. J.; Brady, P. A.; Sanders, J. K. M. *Angew. Chem., Int. Ed.* **1996**, *35*, 2143−2145. (c) Rowan, S. J.; Brady, P. A.; Sanders, J. K. M. *Tetrahedron Lett*. **1996**, *37*, 6013−6016. (d) Rowan, S. J.; Sanders, J. K. M. *J. Org. Chem.* **1998**, *63*, 1536−1546. (e) Brady, P. A.; Sanders, J. K. M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3237−3253. (f) Kaiser, G.; Sanders, J. K. M. *Chem. Commun.* **2000**, 1763−1764.

(3) (a) Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A.; Le Breton, G. C.; Venton, D. L. *Biopolymers* **1996**, *40*, 617−625. (b) Lins, R. J.; Flitsch, S. L.; Turner, N. J.; Irving, E.; Brown, S. A. *Angew. Chem., Int. Ed*. **2002**, *41*, 3405−3407.

(4) (a) Roberts, S. L.; Furlan, R. L. E.; Otto, S.; Sanders, J. K. M. *Org. Biomol. Chem.* **2003**, *1*, 1625−1633. (b) Furlan, R. L. E.; Ng, Y. F.; Cousins, G. R. L.; Redman, J. E.; Sanders, J. K. M. *Tetrahedron* **2002**, *58*, 771−778. (c) Furlan, R. L. E.; Ng, Y. F.; Otto, S.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2001**, *123*, 8876−8877. (d) Cousins, G. R. L.; Furlan, R. L. E.; Ng, Y. F.; Redman, J. E.; Sanders, J. K. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 423−428. (e) Huc, I.; Lehn, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2106−2110. (f) Star, A.; Goldberg. I.; Fuchs, B. *Angew. Chem., Int. Ed*. **2000**, *39*, 2685−2689. (g) Storm, O.; Lüning, U. *Chem.*−*Eur. J*. **2002**, *8*, 793−798. (h) Nazarpack-Kandlousy, N.; Zweigenbaum, J.; Henion, J.; Eliseev, A. V. *J. Comb. Chem.* **1999**, *1*, 199−206. (i) Gousins, G. R. L.; Poulsen, S. A.; Sanders, J. K. M. *Chem. Commun*. **1999**, 1575−1576. (j) Bunyapaiboonsri, T.; Ramström, O.; Lohmann, S.; Lehn, J. M.; Peng, L.; Goeldner, M. *ChemBioChem.* **2001**, *2*, 438−444. (k) Hochgurtel, M.; Kroth, H.; Piecha, D.; Hofmann, M. W.; Nicolau, C.; Krause, S.; Schaaf, O.; Sonnenmoser, G.; Eliseev, A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3382−3387. (l) Kuhnert, N.; Rossignolo, G. M.; Lopez-Periago, A. *Org. Biomol. Chem*. **2003**, *1*, 1157−1170.

(5) Giger, T.; Wigger, M.; Audetat, S.; Benner, S. A. *Synlett* **1998**, 688−692.

(6) (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590−593. (b) Otto, S.; Rurlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc*. **2000**, *122*, 12063−12064. (c) Ramström, O.; Lehn, J. M. *ChemBioChem* **2000**, *1*, 41−48. (d) Hioki, H.; Still, W. C. *J. Org. Chem*. **1998**, *63*, 904−905.

(7) (a) Eliseev, A. V.; Nelen, M. I. *J. Am. Chem. Soc*. **1997**, *119*, 1147−1148. (b) Eliseev, A. V.; Nelen, M. I. *Chem.*−*Eur. J*. **1998**, *4*, 825−834.

(8) (a) Crego-Calama, M.; Hulst, R.; Fokkens, R.; Nibbering, N. M. M.; Timmerman, P.; Reinhoudt, D. N. *Chem. Commun*. **1998**, 1021−1022. (b) Crego-Calama, M.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **2000**, *39*, 755−758. (c) Hof, F.; Nuckolls, C.; Rebek, J. *J. Am. Chem. Soc*. **2000**, *122*, 4251−4252.

(9) (a) Epstein, D. M.; Choudhary, S.; Churchill, R. M.; Keil, K. M.; Eliseev, A. V.; Morrow, J. R. *Inorg. Chem*. **2001**, *40*, 1591−1596. (b) Choudhary, S.; Morrow, J. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 4096−4098. (c) Huc, I.; Krische, M. J.; Funeriu, D. P.; Lehn, J. M. *Eur. J. Inorg. Chem*. **1999**,

1415−1420. (d) Stulz, E.; Ng, Y. F.; Scott, S. M.; Sanders, J. K. M. *Chem. Commun.* **2002**, 524−525. (e) Ziegler, M.; Miranda, J. J.; Andersen, U. N.; Johnson, D. W.; Leary, J. A.; Raymond, K. N. *Angew. Chen., Int. Ed*. **2001**, *40*, 733−736. (f) Albrecht, M.; Blau, O.; Fröhlich, R. *Chem.—Eur. J*. **1999**, *5*, 48−56. (g) Hiraoke, S.; Fujita, M. *J. Am. Chem. Soc*. **1999**, *121*, 10239−10240. (h) Kubota, Y.; Sakamoto, S.; Yamaguchi, K.; Fujita, M. *Proc. Natl. Acad. Sci. U.S.A*. **2002**, *99*, 4854−4856. (i) Sakai, S.; Shigemasa, Y.; Sasaki, T. *Tetrahedron Lett*. **1997**, *38*, 8145−8148. (j) Klekota, B.; Hammond, M. H.; Miller, B. L. *Tetrahedron Lett*. **1997**, *38*, 8639−8642; (k) Klekota, B.; Miller, B. L. *Tetrahedron* **1999**, *55*, 11687−11697. (l) Karan, C.; Miller, B. L. *J. Am. Chem. Soc*. **2001**, *123*, 7455−7456. (m) Case, M. A.; McLendon, G. L. *J. Am. Chem. Soc*. **2000**, *122*, 8089−8090. (n) Constable, E. C.; Housecroft, C. E.; Kulke, T.; Lazzarini, C.; Schofield, E. R.; Zimmermann, Y. *J. Chem. Soc., Dalton Trans*. **2001**, 2864−2871. (o) Goral, V.; Nelen, M. I.; Eliseev, A. V.; Lehn, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1347−1352.

(10) (a) Chung, S. K.; Chang, Y. T. *J. Chem. Soc., Chem. Commun*. **1995**, 13−14. (b) Chung, S. K.; Chang, Y. T.; Ryu, Y. *Pure Appl. Chem*. **1996**, *68*, 931−935.

(11) For synthesis of inositol derivatives, refer to Supporting Information and (a) Khersonsky, S. M.; Chang, Y. T. *Carbohydr. Res*. **2002**, *337*, 75−78. (b) Falshaw, A.; Hart, J. B.; Tyler, P. C. *Carbohydr. Res*. **2000**, *329*, 301−308.

(12) (a) James, T. D.; Sandanayake, S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl*. **1996**, *35*, 1910−1922. (b) Wiecko. J.; Sherman, W. R. *J. Am. Chem. Soc*. **1979**, *101*, 979−983.

(13) (a) Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem*. **1999**, *64*, 3846−3852. (b) Arimori, S.; Bell, M. L.; Oh, C. S.; Frimat, K. A.; James, T. D. *Chem. Commun*. **2001**, 1836−1837. (c) Arimori, S.; Ushiroda, S.; Peter, L. M.; Jenkins, A. T. A.; James, T. D. *Chem. Commun*. **2002**, 2368−2369. (d) Yang, W.; He, H.; Drueckhammer, D. G. *Angew. Chem., Int. Ed*. **2001**, *40*, 1714−1718. (e) Cabell, L. A.; Monahan, M. K.; Anslyn, E. V. *Tetrahedron Lett*. **1999**, *40*, 7753−7756. (f) James, T. D.; Sandanayake, S.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc*. **1995**, *117*, 8982−8987.

(14) Kinetic data showing the accumulation of **7** vs time is available in the Supporting Information.

CC030046Z