Molecular Evolution Using Intramolecular Acyl Migration on *myo*-Inositol Benzoates with Thermodynamic and Kinetic Selectors

Young-Hoon Ahn and Young-Tae Chang*[a]

Abstract: A molecular evolution model was successfully demonstrated by combining the intramolecular acyl migration on inositol tribenzoates and boron selectors. The addition of boric acid to 12 members of DCL (dynamic combinatorial library) induced the dramatic amplification of *myo*-I(2,4,6)Bz_3 (**1**) with up to 94% under thermodynamic (see Figure 1c) control while a portion of phenyl boronic acid caused two significant different distributions: under kinetic control, the pre-equilibrium of DCL shifted to induce the exclusive amplification of 1,4,6-tribenzoyl *myo*-inositol (**7**) with decrease of other members up to 82% from the mixture (see Figure 2b), and changed gradually to form 2,4,6-tribenzoyl *myo*-inositol (**1**) with up to 96% under thermodynamic control (Figure 2c).

Keywords: boronic acid selector · dynamic combinatorial library · intramolecular acyl migration · molecular amplification · molecular evolution

Introduction

Molecular evolution incorporating a dynamic combinatorial library (DCL) has emerged as an approach for the identification and preparation of new host–guest systems.[1] A DCL is composed of members in a reversible equilibrium in which all library members are interconverting, thus providing constant distribution under thermodynamic control. Consequently, a molecular recognition event in the DCL enables the stabilization of the fittest member that shifts the equilibrium toward amplification of the selected member with a consequent decrease in the proportion of poor binders. It requires two key steps for success: 1) continuous DCL generation and 2) effective selection. 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] While some DCLs have successfully demonstrated molecular evolution's proof of concept, there is still a limited number of scaffolds. In fact, most of the efficient DCLs form macrocyclic members.[2,4,6] Scaffold diversification is necessary for a highly efficient and practical system. One of the challenges in this field is a successful carbohydrate dynamic library due to its complexity. There have not been many attempts to generate dynamic combinatorial libraries (DCL) on a carbohydrate scaffold, and the selection method was not effective in giving high yields or selective amplification.[6c,9d] Herein, we report a highly efficient molecular evolution system utilizing intramolecular acyl migration on a carbohydrate scaffold coupled with boric/boronic acid as a selector.

Results and Discussion

Our carbohydrate scaffold is hexahydroxyl cyclohexane (inositol), and we have chosen the most naturally abundant inositol isomer, *myo*-inositol, as the model compound. The polyhydroxyl nature of inositol allows for diverse regioisomers depending upon the position of the attached functional group. Under basic conditions, acyl functionalities on an inositol ring are able to undergo intramolecular migration and generate various regioisomers, while also giving continuous interconversion.[10]

One of the inositol benzoate derivatives, 1,4,5-tribenzoyl *myo*-inositol [**4**, (*myo*-I(1,4,5)Bz_3, Figure 1a], was synthesized (for synthetic procedure, see Supporting Information). The optimized migration condition was sought that would give the least amount of side products, fast generation of all members within a reasonable time, and also with applicability to the selection system. A series of bases were investigated including pyridine, DMAP (4-dimethyl-aminopyridine),

[a] Y.-H. Ahn, Prof. Y.-T. Chang
Department of Chemistry
New York University
New York, NY 10003 (USA)
Fax: (+1) 212-995-4203
E-mail: yt.chang@nyu.edu

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

 DOI: 10.1002/chem.200400234

DABCO (1,4-diazabicyclo[2.2.2]octane), DIEA (*N,N*-diisopropylethlyamine), BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine) on polystyrene, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) and NaOH in aprotic solvents with and without water. We found that increasing amounts of water accelerated benzoyl migration even with mild bases due possibly to the stabilization of the tetrahedral intermediate, but it also accelerated benzoyl group hydrolysis as a side reaction. With the optimized reaction condition (isomer **4** with DBU in anhydrous acetonitrile), we generated all 12 of the expected and possible regioisomers within the DCL and without significant side reactions. The DCL was analyzed by LC-MS, which allowed for the identification of 12 peaks in the chromatogram (Figure 1b); the structure of each isomer was assigned by NMR by using the literature (Scheme 1).[11]

It is well known that boronic acids are able to bind with carbohydrates to form five- or six-membered cyclic esters, as used in carbohydrate recognition.[12] Their binding preference with 1,2 or 1,3-*cis* diols over *trans* diols in sugar systems was appropriate for selection amongst a carbohydrate polyol system. It was also reported that the binding affinity of boronic acid with a sugar depends on the pH of the media.[12a] At pH values lower than the pK_a of boronic acid (acidic conditions), coupling between the boronic acid and a sugar is disfavored, while the coupling is favored at pH values above the pK_a of boronic acid (basic conditions). In our study, boric acid and phenyl boronic acid were investigated for selective binding and amplification.

It was observed that the addition of boric acid to the DCL significantly affects the distribution of the DCL by inducing the amplification of *myo*-I(1,4,6)Bz$_3$ (**1**) with up to approximately 94% (Figure 1c). As the original amount of isomer **1** was about 4% in the pre-equilibrium of the DCL, the enrichment factor is a high 23.5. It was envisioned that boric acid couples with the 1,3,5-trihydroxyl group of *myo*-inositol in a ring-flipped conformation where there are no more available hydroxyl groups. This leads to the isolation of the product from the equilibrium and gives the amplification of **1** (Scheme 1). Once at the final distribution, it remained unchanged over time (Figure 4). This implies that the selection is driven by thermodynamic control. It has been also shown that direct migration from isomer **4** to isomer **1** by adding base and boric acid together without pre-equilibrium resulted in the same final distribution (94% of isomer **1**) (see Figure 5), which again supports the thermodynamic control mechanism.[4a]

Interestingly, when phenyl boronic acid was added to the equilibrium mixture, two significant different stepwise distributions were observed. At first, the pre-equilibrium of DCL was shifted to induce an exclusive amplification of another isomer, *myo*-I(1,4,6)Bz$_3$ (**7**) with up to 82% (Figure 2b) as

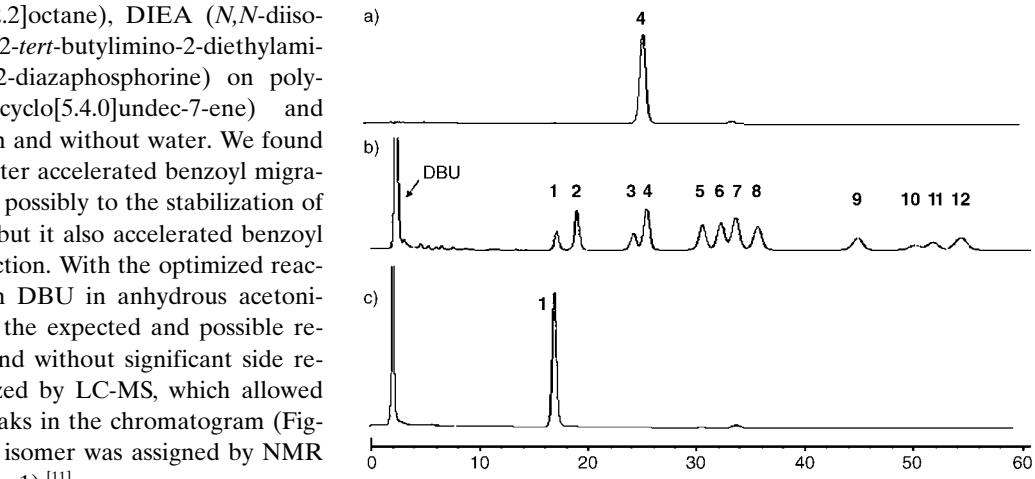
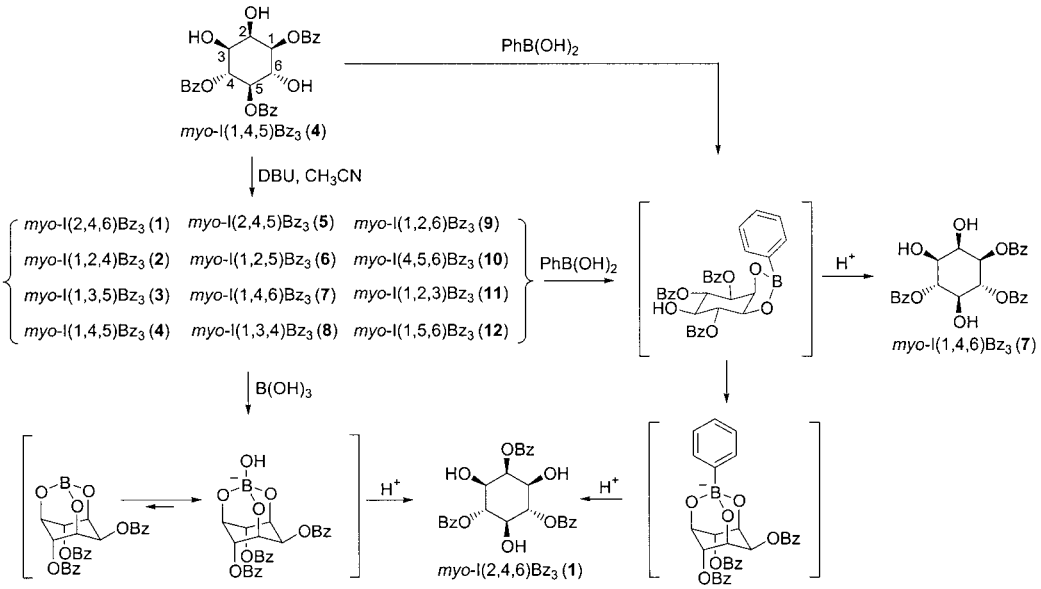


Figure 1. Amplification chromatogram using $B(OH)_3$ a) HPLC chromatogram (C18 column: 4.6×150 mm, eluted with a solution of water, acetonitrile, and methanol in a ratio of 52:18:30) of I(1,4,5)Bz$_3$ (**4**), b) DCL at 1.5 h after addition of DBU, and c) 72 h after addition of boric acid (94% of **1**).



Scheme 1. Library members generated by intramolecular acyl migration and two types of selection using boric acid and phenyl boronic acid.

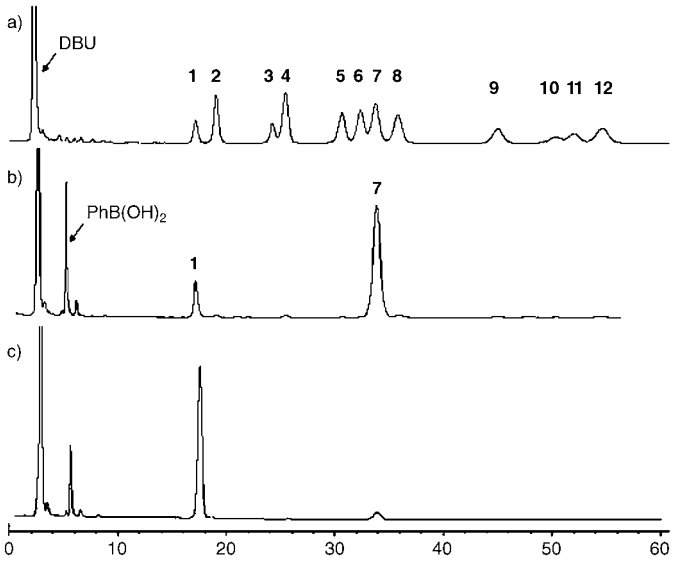


Figure 2. Amplification chromatogram using $PhB(OH)_2$ a) chromatogram of a 12 member DCL in pre-equilibrium by DBU, b) 5 h after the addition of $PhB(OH)_2$ (82% of **7**), c) 205 h after the addition of $PhB(OH)_2$ (96% of **1**).

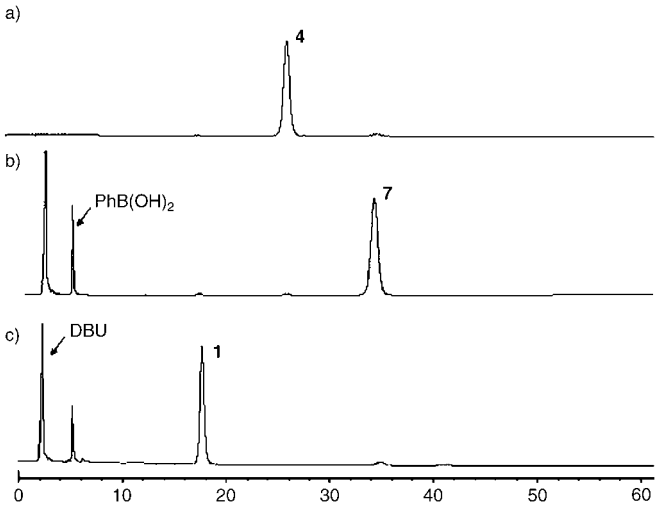


Figure 3. Direct migration chromatogram using $PhB(OH)_2$ a) The HPLC chromatogram of $I(1,4,5)Bz_3$ (**4**), b) 25 min after addition of DBU and $PhB(OH)_2$ (98% of **7**), c) after an additional 124 h (96% of **1**).

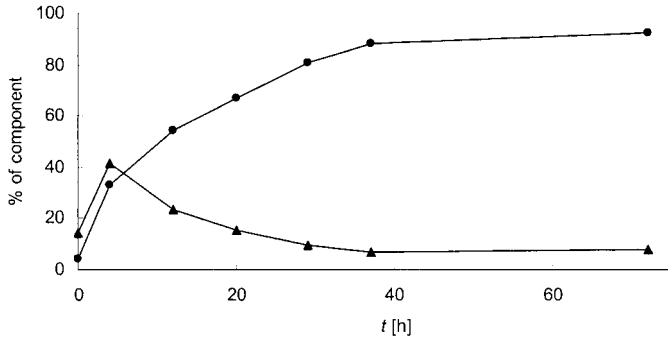


Figure 4. Amplification of **1** using boric acid after pre-equilibrium. Reaction conditions: $I(1,4,5)Bz_3$ (**4**) (1 mg), DBU (20 μL, 66 equiv) in acetonitrile (20 mL) for 1.5 h, and then boric acid (2 mg, 15 equiv) where $t=0$ at that point, ●: $I(2,4,6)Bz_3$ (**1**), ▲: $I(1,4,6)Bz_3$ (**7**).

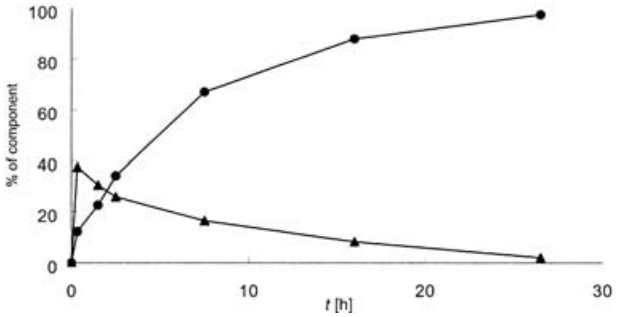


Figure 5. Direct migration to **1** using boric acid without pre-equilibrium. Reaction conditions: $I(1,4,5)Bz_3$ (**4**) (1 mg), boric acid (2 mg, 15 equiv) in acetonitrile (1 mL), and then DBU (20 μL, 66 equiv) where $t=0$ at that point, ●: $I(2,4,6)Bz_3$ (**1**), ▲: $I(1,4,6)Bz_3$ (**7**).

the kinetic product, and changed gradually to form the thermodynamic isomer (**1**) up to 96% (Figure 2c). It is envisioned that phenyl boronic acid coupled quickly (kinetically) with the 1,2-*cis* diols of the DCL members, whenever available during the acyl migration, to form a stable five-membered ring. Even though there are four possible regioisomers with 1,2-*cis* coupling (compound **4**, **7**, **10**, and **12**), the 5-benzoyl group seemed to have the most severe steric hindrance against phenyl ring of 1,2-*cis*-boronic ester, and thus isomer **7**, *myo*-$I(1,4,6)Bz_3$, was kinetically favored and accumulated (Scheme 1). When the reaction proceeds longer, phenyl boronic acid is able to accept three hydroxyl groups to form an even more stable complex giving the thermodynamic isomer **1**, as in the case of the boric acid selector (Figure 6). Also, we investigated the direct migration from *myo*-$I(1,4,5)Bz_3$ (**4**) without pre-equilibrium, in which the acyl migration occurred in the presence of phenyl boronic acid. Absolute amplification of the kinetic product, **7**, with up to 98%, was obtained initially (see Figure 3b), with slow transformation to the thermodynamic product **1** over time (Figures 3c and 7).

The practical use of this concept beyond the analytical scale was performed on a 30 mg scale, purifying the thermodynamic and kinetic products from the reaction mixture, respectively. The purification of the thermodynamic product

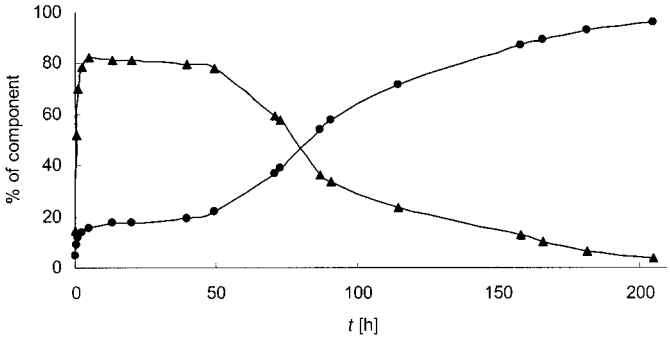


Figure 6. Amplification of **1** and **7** using phenyl boronic acid after pre-equilibrium. Reaction conditions: $I(1,4,5)Bz_3$ (**4**) (1 mg), DBU (20 μL, 66 equiv) in acetonitrile (20 mL) for 1.5 h. Phenyl boronic acid (3.7 mg, 15 equiv) was added and then concentrated to 1 mL where $t=0$ at that point. Additional DBU (10 μL, 33 equiv) was added at 55 h to accelerate the equilibrium, ●: $I(2,4,6)Bz_3$ (**1**), ▲: $I(1,4,6)Bz_3$ (**7**).

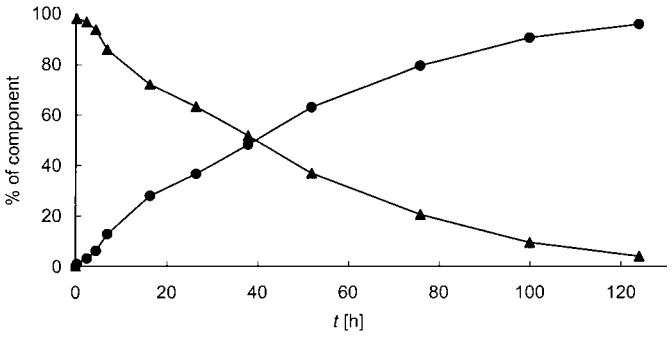


Figure 7. Direct migration to **7** and **1** using phenyl boronic acid without pre-equilibrium. Reaction conditions: I(1,4,5)Bz_3 (**4**) (1 mg), phenyl boronic acid (3.7 mg, 15 equiv) in acetonitrile (1 mL), and then DBU (20 μL, 66 equiv) where $t=0$ at that point, ●: I(2,4,6)Bz_3 (**1**), ▲: I(1,4,6)Bz_3 (**7**).

(**1**) on silica gel after its amplification of up to 94% afforded the 83% recovery of **1**. The isolation of kinetic product (**7**) that is amplified with up to 98% from the direct migration without pre-equilibrium led to the 92% recovery of **7**.

Conclusion

We demonstrated highly efficient molecular evolution on a *myo*-inositol model by combining intramolecular acyl migration and both kinetic and thermodynamic selectors. We believe that intramolecular acyl migration is applicable to an even large number of dynamic carbohydrate libraries, and if coupled with a broad range of selectors, this method will open a wide range of applications.

Experimental Section

Materials and general methods: All reactions were performed in oven-dried glassware under positive nitrogen pressure. Unless otherwise noted, starting materials and solvents were purchased from Aldrich and Acros organics and used without purification. Analytical TLC was carried out on Merck 60 F254 silica gel plate (0.25 mm layer thickness) and visualization was done with UV light, and/or by spaying with a 5% solution of phosphomolybdic acid followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (230–400 mesh). ^{1}H NMR (200 MHz) spectra were determined on Varian Genini 200 spectrometer. Chemical shifts were reported in parts per million (ppm) relative to internal standard as tetramethylsilane, and coupling constants (J) are in Hertz (Hz). All compounds were identified by LC-MS (Agilent Technologies) using a C18 column (4.6×150 mm) with 65 minutes of elution time using a solution of H_2O/CH_3CN/MeOH in a ratio of 52:18:30 (containing 0.1% acetic acid) with a UV detector at $\lambda=250$ and 230 nm and an electrospray ionization source.

Molecular amplification experiment with pre-equilibrium: DBU (20 μL, 0.13 mmol) was added at room temperature to a solution of **4** (1 mg, 2.0 μmol) in acetonitrile (20 mL). After the reaction mixture was stirred for 1.5 h, the full migration of 12 isomers was confirmed by HPLC-MS. A solution of boric acid (2 mg, 0.032 mmol) in DMSO (50 μL) or phenyl boronic acid (3.75 mg, 0.032 mmol) was added to the reaction mixture. The change of the distribution was analyzed by HPLC-MS.[13]

Molecular amplification experiment without pre-equilibrium: Boric acid (2 mg, 0.032 mmol) in DMSO (50 μL) or phenyl boronic acid (3.75 mg, 0.032 mmol), and DBU (20 μL, 0.13 mmol) were added at room temperature to a solution of **4** (1 mg, 2 mmol) in acetonitrile (1 mL). The reaction mixture was analyzed by HPLC-MS.

Preparation of the thermodynamic product (1): DBU (600 μL, 3.9 mmol) was added to a solution of **4** (30 mg, 0.061 mmol) in acetonitrile (600 mL). After the reaction mixture was stirred for 1.5 h, boric acid (60 mg, 0.96 mmol) in DMSO (0.5 mL) was added to the reaction mixture. To accelerate the rate of amplification, the reaction mixture was concentrated to about 60 mL, and stirred for 2 d. The reaction mixture was quenched by acetic acid (1 mL), diluted with ethyl acetate, washed with 1 N HCl, aq. $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, concentrated, and purified by chromatography on silica gel (EtOAc/Hex 1:1) to give **1** (25 mg, 83%). R_f = 0.30 (EtOAc/Hex 1:1); ^{1}H NMR (CD_3OD): δ = 3.89 (t, J=9.5 Hz, 1H), 4.00 (dd, J=2.9, 10.0 Hz, 2H), 5.57 (t, J=9.7 Hz, 2H), 5.77 (t, J=2.8 Hz, 1H), 7.26–8.15 (m, 15H); LC-MS: m/z: 493 $[M+H]^+$, 475 $[M-H_2O+H]^+$.

Preparation of the kinetic product (7): Phenyl boronic acid (113 mg, 0.96 mmol), and DBU (600 μL, 3.9 mmol) was added to a solution of **4** (30 mg, 0.061 mmol) in acetonitrile (30 mL). After the solution was stirred for 1 h, the reaction mixture was quenched by acetic acid (1 mL), and diluted with ethyl acetate, and washed with 1 N HCl, aq. $NaHCO_3$, and brine solution. The resulting organic layer was dried over $MgSO_4$, concentrated, and purified by chromatography on silca gel (EtOAc/Hex 1:2) to give **7** (27.5 mg, 92%). R_f = 0.37 (EtOAc/Hex 1:1); ^{1}H NMR (CD_3OD): δ = 4.04 (dd, J=2.4, 10.1 Hz, 1H), 4.34 (t, J=2.3 Hz, 1H), 4.48 (t, J=10.0 Hz, 1H), 5.13 (dd, J=2.4, 10.0 Hz, 1H), 5.49 (dd, J=9.3, 10.0 Hz, 1H), 5.80 (t, J=10.0 Hz, 1H), 7.32–8.11 (m, 15H); LC-MS: m/z: 493 $[M+H]^+$.

Acknowledgement

This work was supported by the Petroleum Research Fund from the American Chemical Society.

[1] a) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **2002**, *6*, 321–327; b) S. J. Rowan, S. J. Cantrill, G. R. L. Cousins, J. K. M. Sanders, J. F. Stoddart, *Angew. Chem.* **2002**, *114*, 938–993; *Angew. Chem. Int. Ed.* **2002**, *41*, 898–952; c) J. M. Lehn, A. V. Eliseev, *Science* **2001**, *291*, 2331–2332; d) C. Karan, B. L. Miller, *Drug Discovery Today* **2000**, *5*, 67–75.

[2] a) P. A. Brady, R. P. Bonar-Law, S. J. Rowan, C. J. Suckling, J. K. M. Sanders, *Chem. Commun.* **1996**, 319–320; b) P. A. Brady, J. K. M. Sanders, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3237–3253; c) G. Kaiser, J. K. M. Sanders, *Chem. Commun.* **2000**, 1763–1764.

[3] R. J. Lins, S. L. Flitsch, N. J. Turner, E. Irving, S. A. Brown, *Angew. Chem.* **2002**, *114*, 3555–3557; *Angew. Chem. Int. Ed.* **2002**, *41*, 3405–3407.

[4] a) S. L. Roberts, R. L. E. Furlan, S. Otto, J. K. M. Sanders, *Org. Biomol. Chem.* **2003**, *1*, 1625–1633; b) R. L. E. Furlan, Y. F. Ng, S. Otto, J. K. M. Sanders, *J. Am. Chem. Soc.* **2001**, *123*, 8876–8877; c) G. R. L. Cousins, R. L. E. Furlan, Y. F. Ng, J. E. Redman, J. K. M. Sanders, *Angew. Chem.* **2001**, *113*, 437–442; *Angew. Chem. Int. Ed.* **2001**, *40*, 423–428; d) A. Star, I. Goldberg. B. Fuchs, *Angew. Chem.* **2000**, *112*, 2797–2801; *Angew. Chem. Int. Ed.* **2000**, *39*, 2685–2689; e) O. Storm, U. Lüning, *Chem. Eur. J.* **2002**, *8*, 793–798.

[5] T. Giger, M. Wigger, S. Audetat, S. A. Benner, *Synlett* **1998**, 688–692.

[6] a) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Science* **2002**, *297*, 590–593; b) S. Otto, R. L. E. Rurlan, J. K. M. Sanders, *J. Am. Chem. Soc.* **2000**, *122*, 12063–12064; c) O. Ramström, J. M. Lehn, *ChemBioChem* **2000**, *1*, 41–48.

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

[8] a) M. Crego-Calama, P. Timmerman, D. N. Reinhoudt, *Angew. Chem.* **2000**, *112*, 771–774; *Angew. Chem. Int. Ed.* **2000**, *39*, 755–758; b) F. Hof, C. Nuckolls, J. Rebek, *J. Am. Chem. Soc.* **2000**, *122*, 4251–4252.

[9] a) S. Choudhary, J. R. Morrow, *Angew. Chem.* **2002**, *114*, 4270–4272; *Angew. Chem. Int. Ed.* **2002**, *41*, 4096–4098; b) E. Stulz, Y. F. Ng,

S. M. Scott, J. K. M. Sanders, *Chem. Commun.* **2002**, 524 – 525; c) M. Ziegler, J. J. Miranda, U. N. Andersen, D. W. Johnson, J. A. Leary, K. N. Raymond, *Angew. Chem.* **2001**, *113*, 755 – 758; *Angew. Chem. Int. Ed.* **2001**, *40*, 733 – 736; d) S. Sakai, Y. Shigemasa, T. Sasaki, *Tetrahedron Lett.* **1997**, *38*, 8145 – 8148; e) C. Karan, B. L. Miller, *J. Am. Chem. Soc.* **2001**, *123*, 7455 – 7456; f) M. A. Case, G. L. McLendon, *J. Am. Chem. Soc.* **2000**, *122*, 8089 – 8090; g) V. Goral, M. I. Nelen, A. V. Eliseev, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1347 – 1352.

[10] S. K. Chung, Y. T. Chang, *J. Chem. Soc. Chem. Commun.* **1995**, 13 – 14.

[11] S. K. Chung, Y. T. Chang, K. H. Sohn, *J. Chem. Soc. Chem. Commun.* **1996**, 163 – 164; b) S. K. Chung, Y. T. Chang, Y. Ryu, *Pure Appl. Chem.* **1996**, *68*, 931 – 935.

[12] a) T. D. James, S. Sandanayake, S. Shinkai, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1910 – 1922; b) J. Wiecko. W. R. Sherman, *J. Am. Chem. Soc.* **1979**, *101*, 979 – 983; c) H. Eggert, J. Frederiksen, C. Morin, J. C. Norrild, *J. Org. Chem.* **1999**, *64*, 3846 – 3852; d) W. Yang, H. He, D. G. Drueckhammer, *Angew. Chem.* **2001**, *113*, 1764 – 1768; *Angew. Chem. Int. Ed.* **2001**, *40*, 1714 – 1718; e) T. D. James, S. Sandanayake, R. Iguchi, S. Shinkai, *J. Am. Chem. Soc.* **1995**, *117*, 8982 – 8987.

[13] The concentrated reaction mixture led to intermolecular benzoyl migration as a side reaction, which could be suppressed by dilution (20 times of direct migration). However, in the case of phenyl boronic acid, the reaction mixture was concentrated to 1 mL in order to accelerate the rate of the amplification since it was found that the addition of boric acid or phenyl boronic acid stopped intermolecular benzoyl migration from going further.

Received: March 11, 2004
Published online: May 27, 2004

