New Asymmetric Synthesis of α-Aminoboronic Acids Containing Functionalized Side Chains

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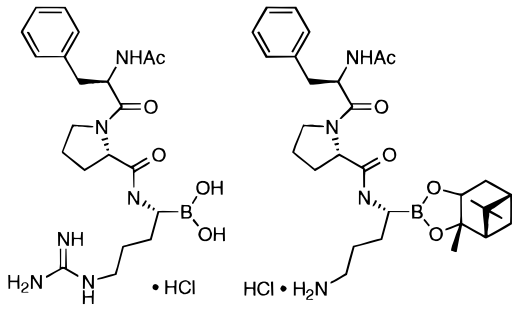
Introduction

Thrombin is the last serine protease in the blood coagulation cascade and is responsible for activation of fibrinogen to fibrin and for clot formation. Thrombin plays a key role in pathological thrombotic conditions of deep vein thrombosis, pulmonary embolus, myocardial infarction, unstable angina, and stroke. Thus, thrombin inhibition is an important tool for the treatment of these conditions. Peptides, such as DuP 714 (**1**), containing boroarginine at the primary residue are potent thrombin inhibitors.[1] Boropeptides inhibit serine proteases by forming a tetrahedral -ate complex with serine proteases.[2] Previous synthetic methods for the economical large-scale synthesis of boroarginine-containing thrombin inhibitors are limited by numerous drawbacks such as use of expensive reagents, use of extremely low temperatures, nonstereoselective steps, cyclization to undesired boroproline, and use of azides.[1,3] In this paper, we describe an acetal-based anion addition approach which solves the above drawbacks and is amenable to large-scale synthesis.

Results and Discussion

The synthesis of boropeptide **2** containing basic α-amino boronic ester relies on the asymmetric induction via the addition of a suitable anion to the dichloromethylboronic ester **3a**−**c** containing a C_2 symmetric diol as a chiral auxiliary. In previous studies,[4] we found that ketal functionalized boropeptides are easily converted to desired amine by appropriate manipulation at the end of the synthesis. Therefore, we used Grignard anions containing acetal side chains such as **4** for developing a large scale route for synthesis of boroornithine- and boroarginine-containing thrombin inhibitors.

Matteson previously reported the use of C_2 symmetric diols for chiral induction and the synthesis of α-chloro boronic esters.[5] We utilized this approach for the asymmetric synthesis of boroornithine **2**. The Grignard reagent **4** served as the anion and was formed at temperatures less that 40 °C to prevent formation of undesired Wurtz coupling product.[6] Addition of the Grignard **4** to dichloromethyl boronic ester of different C_2 symmetric diols **3a**−**c** possessing varying degrees of steric hindrance proceeded at −78 °C with different degrees of asymmetric induction (Scheme 1). The diastereomeric excess is established by transesterifying the chlorides **5a**−**c** with (+)-pinanediol to provide boronic ester **6** (64% overall yield) and measuring the ratio of the "endo"-pinanediol proton of the two diastereomers at 400 MHz. From this point in the synthesis the boronic esters are handled as pinanediol esters due to good stability during chemical synthesis.



DuP 714, **1** **2**

The addition of Grignard **4** to boronic ester **3a** derived from (*R*,*R*)-2,3-butanediol, **3b** derived from (*R*,*R*)-diphenylethanediol (*R*,*R*-DIPED), and **3c** derived from (*R*,*R*)-dicyclohexaneethanediol[7] (*R*,*R*-DICHED) provided the desired (*S*)-chloride **6** in 82%, 78%, and >99% de, respectively. Temperature optimization studies indicate that the dioxolane Grignard **4** can be added to (*R*,*R*)-DICHED dichloromethylboronic ester **3c** at −60 °C, −40 °C, and −20 °C with no change in the diastereomeric excess (Table 1).

The chloride **6** is treated with LHMDS (Scheme 2) to give S_N2 stereoinversion and provide disilazane **7** (99% yield). Treatment of compound **7** with 3 equiv of anhydrous HCl in ether provided amine **8** as the HCl salt (99% yield). Boropeptide **10** is obtained (80% yield) by coupling amine **8** with dipeptide **9** using mixed anhydride conditions with isobutyl chloroformate (IBCF), *N*-methylmorpholine, and triethylamine.[8] Attempts to deprotect acetal **10** (Scheme 3) using TFA/acetone resulted in cyclization to the hemiaminal **11**. A precedent for this cyclization is in the literature for a similar nonboronic acid peptide.[9] Attempts to convert compound **11** to boroornithine **2** by reductive amination with ammonium acetate and sodium cyanoborohydride were unsuccessful. The acetal **10** was resistant to hydrolysis under numerous other acidic conditions. Treatment of acetal **10** with hydroxylamine HCl in refluxing ethanol[10] provided mixture of oxime **12** and nitrile **13** (88% conversion). The mixture of **12** and **13** are separated using HPLC for structural characterization. Hydrogenation of the mixture of **12** and **13**

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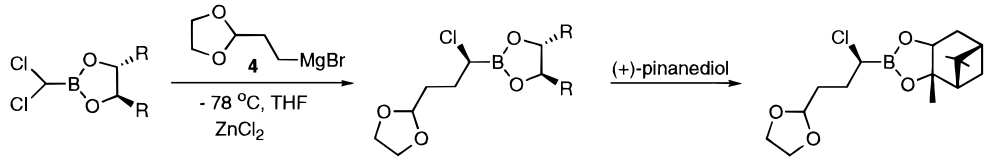
Scheme 1



3a R = -CH_3
3b R = -C_6H_5
3c R = -C_6H_{11}

5a R = -CH_3
5b R = -C_6H_5
5c R = -C_6H_{11}

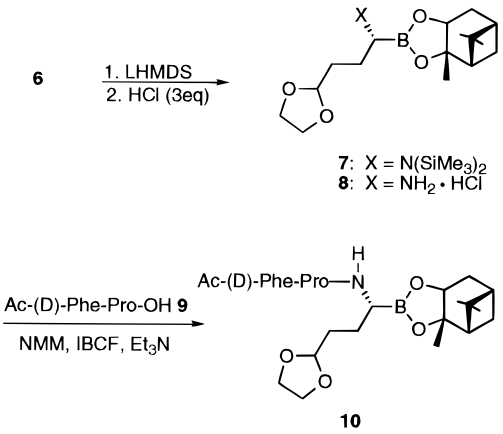
6

Table 1. Effect of Diol Substituent in Reactant 3a−c and Reaction Temperature[a] on Diastereomeric Ratios Obtained for Product 6

diol	boronic ester	temperature	% de
(*R*,*R*)-2,3-butanediol	**3a**	−78 °C	82
(*R*,*R*)-DIPED	**3b**	−78 °C	78
(*R*,*R*)-DICHED	**3c**	−78 °C	>99
(*R*,*R*)-DICHED	**3c**	−60 °C	>99
(*R*,*R*)-DICHED	**3c**	−40 °C	>99
(*R*,*R*)-DICHED	**3c**	−20 °C	>99

[a] Temperature at which dichloromethyl boronic ester **3a−c** is maintained during addition of Grignard reagent **4**.

Scheme 2



(Scheme 4) with Pearlman's catalyst provides the desired boroornithine derivative **2** (80% yield). The boroornithine **2** is converted to boroarginine **14** (65%) using a previously published procedure of formamidine sulfonic acid and DMAP in EtOH at 78 °C.[3] Pinanediol boronic ester **14** is converted to free boronic acid DuP 714 (**1**) (Scheme 5) by transesterification of the pinanediol boropeptide **14** with phenyl boronic acid in an ether:water biphasic system.[3,11] DuP 714 (**1**) is readily soluble in water while both phenylboronic acid **15** and pinanediol phenylboronate ester **16** are ether soluble. Thus, the solubility difference of the boropeptide boronic acid **1** and pinanediol phenylboronate **16** in the two immiscible phases of ether and water drive the transesterification to completion.

In conclusion, we have developed a new method for the large-scale synthesis of boroornithine **2** in 35% overall yield under conditions amenable for large scale synthesis. Additional advantages of this route include high asymmetric induction at temperatures readily achievable on a large scale (−20 °C) and elimination of intermediates that form boroproline side products.

Experimental Section

General Methods. All reactions were carried out under a positive pressure of dry nitrogen and anhydrous conditions unless otherwise stated. Solvents and reagents were used as purchased from Aldrich Chemical Co. Analytical and preparative scale HPLC were done using Vydac C4 columns. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 400 MHz.

[3a*S*-[2(*R),3aα,4*β*,6*β*,7a*β*]]-2-[1-Chloro-3-(1,3-dioxolan-2-yl)propyl]hexahydro-5,5-dimethyl-4,6-methano-1,3,2-benzodioxaborole (6).** Dioxolane magnesium bromide **4** was prepared by the method of Büchi and Wüest[6a] and added to (*R*,*R*)-1,2-dicyclohexylethanediol dichloromethyl boronic ester (**3c**) (2.99 g, 9.38 mmol) in 30 mL of anhydrous THF at −20 °C. After addition of **4**, the reaction was stirred for 5 min and treated with 10 mL of 1.0 M zinc chloride solution in ether. The reaction was warmed to room temperature, stirred overnight, and treated with (+)-pinanediol (1.59 g, 9.38 mmol). After 30 min, the reaction was worked up by extraction with 1:6 ethyl acetate: hexanes and saturated ammonium chloride solution. The organics were concentrated and the product was purified using column chromatography by eluting with 1:7 ethyl acetate: hexanes to provide 1.98 g (64%) of colorless liquid **6**: ^{1}H-NMR ($CDCl_3$) δ 4.90 (t, J = 4.4 Hz, 1H), 4.36 (dd, J = 1.8, 9.2 Hz, 1H), 3.97 (m, 2H), 3.85 (m, 2H), 3.53 (dd, J = 5.9, 8.1 Hz, 1H), 2.39−2.21 (m, 2H), 2.09 (t, J = 5.1 Hz, 1H), 2.05−1.78 (m, 6H), 1.41 (s, 3H), 1.29 (s, 3H), 1.23 (d, J = 19.4 Hz, 1H), 0.84 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ 103.8, 86.5, 78.3, 64.7, 64.7, 51.0, 42.6 (br), 39.1, 38.0, 35.0, 31.3, 28.3, 28.2, 26.8, 26.1, 23.7; IR (neat) 2926, 1386, 1140 cm^{-1}; HRMS calcd for (M + H)$^+$: $C_{16}H_{30}NO_4ClB$ 329.168951, found 329.169093.

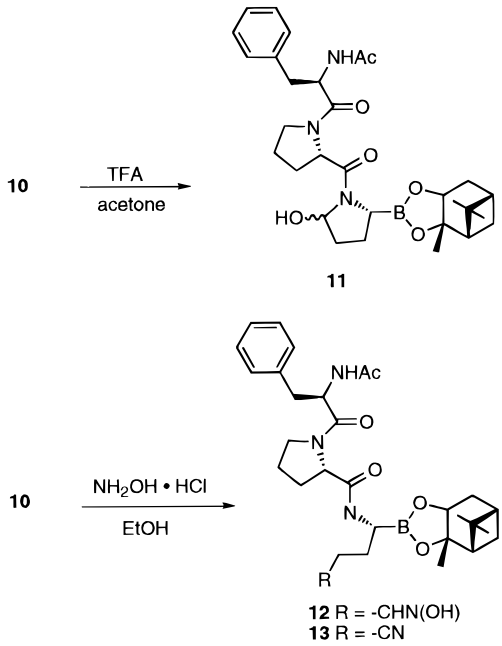
[3a*S*-[2(*S),3aα,4*β*,6*β*,7a*β*]]-α-[2-(1,3-Dioxolan-2-yl)ethyl]-hexahydro-5,5-dimethyl-*N*,*N*-bis(trimethylsilyl)-4,6-methano-1,3,2-benzodioxaborole-2-methanamine (7).** LHMDS (16.7 mmol) was cooled to −78 °C and treated with α-chloroboronic ester **6** (4.40 g, 13.4 mmol) dissolved in 60 mL of anhydrous THF. The reaction was warmed to room temperature and stirred overnight. The organic solvents were removed by evaporation to provide a white solid. Byproduct lithium chloride was filtered from an ethereal solution of the crude product. The ether was evaporated to provide 6.06 g (99%) of a colorless viscous liquid **7**: ^{1}H-NMR ($CDCl_3$) δ 4.74 (t, J = 4.64 Hz, 1H), 4.17 (dd, J = 1.96, 8.8 Hz, 1H), 3.85 (m, 2H), 3.73 (m, 2H), 2.46 (t, J = 6.84 Hz, 1H), 2.23−2.05 (m, 2H), 1.91 (t, J = 5.1 Hz, 1H), 1.80−1.41 (m, 6H), 1.26 (s, 3H), 1.17 (s, 3H), 1.01 (d, J = 10.7 Hz, 1H), 0.72 (s, 3H), 0.10 (s, 18H); ^{13}C-NMR ($CDCl_3$) δ 105.1, 85.4, 78.3, 64.8, 51.5, 42.5, 39.5, 38.2, 35.4, 32.3, 29.7, 28.4, 27.1, 26.3, 24.0, 2.9; IR (neat) 2924, 1378, 922 cm^{-1}; HRMS calcd for (M + H)$^+$: $C_{22}H_{44}NO_4Si_2B$ 454.29766, found 454.298021.

[3a*S*-[2(*S),3aα,4*β*,6*β*,7a*β*]]-α-[2-(1,3-Dioxolan-2-yl)ethyl]-hexahydro-5,5-dimethyl-4,6-methano-1,3,2-benzodioxaborole-2-methanamine (8·HCl).** Hexamethyldisilazane **7** (6.06 g, 13.4 mmol) in 60 mL of anhydrous ether was cooled to −78 °C and treated with 1 M solution of hydrogen chloride in ether (50 mL) and warmed up to room temperature. The solution was stirred overnight, and the solvents were evaporated to provide 4.62 g (100%) of white solid **8**: ^{1}H-NMR (CD_3OD) δ 4.78 (t, J = 3.9 Hz, 1H), 4.41 (dd, J = 1.96, 9.0 Hz, 1H), 3.89 (m, 2H), 3.78 (m, 2H), 2.87 (t, J = 7.08 Hz, 1H), 2.35 (m, 1H), 2.31 (m, 1H), 2.01 (t, J = 5.3 Hz, 1H), 1.88−1.73 (m, 6H), 1.39 (s, 3H), 1.26 (s, 3H), 1.09 (d, J = 10.9 Hz, 1H) 0.82 (s, 3H); ^{13}C-NMR (CD_3OD) δ 104.7, 89.0, 80.2, 66.0, 51.5, 40.8, 39.3, 38.4 (br), 36.0, 31.5, 28.9, 27.4, 27.3, 24.8, 24.3; HRMS calcd for (M + H)$^+$: $C_{16}H_{28}NO_4B$ 310.218964, found 310.219296.

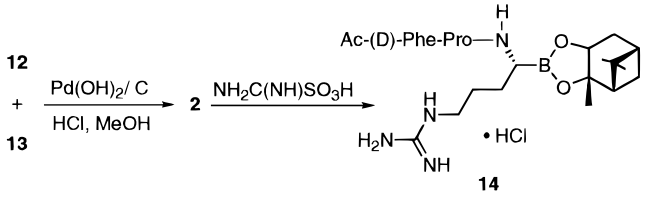
[3a*S*-[2(*S),3aα,4*β*,6*β*,7a*β*]]-*N*-Acetyl-D-phenylalanyl-*N*-[3-(1,3-dioxolan-2-yl)-1-(hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)propyl]-L-prolinamide (10).** The peptide was synthesized from dipeptide **9** and α-aminoboronic acid **8** using the mixed anhydride coupling

(11) Kettner, C. A. U.S. Patent 5,384,410, 1995.

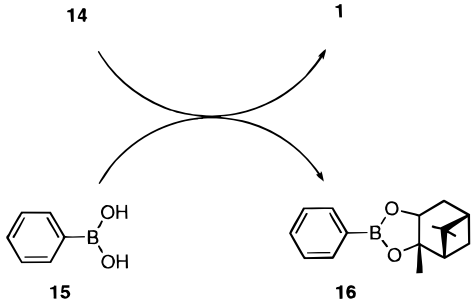
Scheme 3



Scheme 4



Scheme 5



method.[8] The crude solid obtained was subjected to column chromatography using silica gel and eluting with 1:9 MeOH:$CHCl_3$ to provide 2.76 g (80%) of **10** as a white solid: ^{1}H-NMR (CD_3OD) δ 7.32−7.22 (m, 5H), 4.79 (t, J = 4.64 Hz, 1H), 4.58 (dd, J = 6.6, 9.3 Hz, 1H), 4.48 (dd, J = 2.68, 8.05 Hz, 1H), 4.15 (dd, J = 2.4, 8.8 Hz, 1H), 3.91 (m, 2H), 3.79 (m, 2H), 3.30 (m, 1H), 2.98 (m, 2H), 2.66 (m, 1H), 2.54 (dd, J = 6.1, 8.3 Hz, 1H), 2.33−2.27 (m, 1H), 2.14−2.08 (m, 1H), 1.98 (s, 3H), 1.96−1.91 (m, 2H), 1.87−1.73 (m, 6H), 1.73−1.46 (m, 3H), 1.43 (d, J = 10.5 Hz, 1H), 1.32 (s, 3H), 1.27 (s, 3H), 0.85 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ 177.3, 173.8, 173.1, 137.4, 130.5, 129.7, 128.3, 105.7, 84.3, 77.5, 65.9, 65.8, 58.6, 55.2, 53.6, 48.0, 44.3 (br), 41.4, 39.2, 38.3, 37.6, 32.9, 29.8, 29.7, 27.8, 27.5, 26.6, 24.9, 24.6, 22.2; IR (KBr pellet) 3292, 2928, 1638, 1450 cm^{-1}; HRMS calcd for (M + H)$^+$: $C_{32}H_{46}N_3O_7B$ 596.350707, found 596.351059.

[3a*S*-[2(*S),3aα,4β,6β,7aβ]]-*N*-Acetyl-D-phenylalanyl-*N*-[1-(hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-(hydroxyimino)butyl]-L-prolinamide (12).** Peptide ketal **10** (350 mg, 0.59 mmol) dissolved in 30 mL of absolute ethanol was treated with hydroxylamine hydrochloride (53 mg, 0.77 mmol) and heated at 80 °C (reflux) for 2.5 h. After cooling, the solvent was evaporated and the product was extracted with 75 mL of ethyl acetate and washed with 10 mL of water, concentrated, and purified using silica gel chromatography and 1:9 MeOH:$CHCl_3$ as eluant to provide a white solid (88%) containing a mixture of oxime **12** and nitrile **13**. The oxime **12** and nitrile **13** were characterized separately after HPLC purification using Vydac C4 column and 70:30 MeOH:H_2O as eluant: ^{1}H-NMR (CD_3OD) δ (*cis* and *trans* diastereomers) 7.35 (t, J = 5.86 Hz, 1H *cis* and *trans* isomer) 7.25 (m, 5H), 6.64 (t, J = 5.37 Hz, 1H *cis* and *trans* isomer), 4.61 (m, 1H), 4.48 (m, 1H), 4.17 (m, 1H), 3.65 (m, 1H), 2.99 (m, 2H), 2.67−2.53 (m, 2H), 2.42 (m, 1H), 2.39−2.12 (m, 3H), 1.96 (s, 3H), 1.87−1.54 (m, 9H), 1.43 (m, 1H), 1.33 (s, 3H), 1.28 (s, 3H), 0.86 (s, 3H); ^{13}C-NMR (CD_3OD) δ 177.4, 173.6, 173.1, 152.7, 152.0, 137.4, 130.5, 129.7, 128.3, 84.4, 77.5, 58.7, 55.2, 53.5, 48.0, 44.2 (br), 41.4, 39.2, 38.4, 37.6, 29.9, 29.7, 29.3, 28.7, 28.4, 27.8, 27.5, 24.9, 24.6, 24.3, 22.2; IR 3280, 2926, 2420, 1638, 1450, 938 cm^{-1}; HRMS calcd for (M + H)$^+$: $C_{30}H_{43}N_4O_6B$ 567.33622, found 567.335391.

[3a*S*-[2(*S),3aα,4β,6β,7aβ]]-*N*-Acetyl-D-phenylalanyl-*N*-[3-cyano-1-(hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)propyl]-L-prolinamide (13):** ^{1}H-NMR (CD_3OD) δ 7.34−7.23 (m, 5H), 4.55 (dd, J = 6.2, 9.2 Hz, 1H), 4.48 (dd, J = 3.3, 7.7 Hz, 1H), 4.17 (dd, J = 2.2, 8.4 Hz, 1H), 3.65 (m, 1H), 3.00 (m, 2H), 2.68−2.55 (m, 3H), 2.55−2.30 (m, 2H), 2.15 (m, 1H), 1.99 (s, 3H), 1.97−1.68 (m, 8H), 1.60 (m, 1H), 1.40 (d, J = 10.25 Hz, 1H), 1.33 (s, 3H), 1.28 (s, 3H), 0.86 (s, 3H); ^{13}C-NMR (CD_3OD) δ 176.6, 172.2, 171.9, 135.8, 129.0, 128.2, 126.9, 119.8, 90.2, 83.1, 76.1, 57.3, 53.9, 52.0, 39.8, 37.6, 36.7, 36.2, 28.3, 28.1, 26.8, 26.2, 26.0, 23.4, 23.0, 20.7, 13.4; IR 3330, 2926, 2242, 1602 cm^{-1}; HRMS calcd for (M + H)$^+$: $C_{30}H_{41}N_4O_5B$ 549.324188, found 549.324826.

[3a*S*-[2(*S),3aα,4β,6β,7aβ]]-*N*-Acetyl-D-phenylalanyl-*N*-[4-amino-1-(hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)butyl]-L-prolinamide (2·TFA).** A mixture of oxime **12** and nitrile **13** (104 mg) in methanol (20 mL), a solution of HCl in ether (1 M, 0.2 mL), and Pearlman's catalyst (250 mg) was hydrogenated at 1 atm. Upon completion (TLC monitoring), the catalyst was filtered through Celite and solvent was removed to provide peptide **2** as white solid (104 mg, 80% yield). The product was purified with HPLC using Vydac C4 column and 80:20 MeOH:H_2O containing 0.1% TFA as eluant: ^{1}H-NMR (CD_3OD) δ 7.34−7.22 (m, 5H), 4.55 (dd, J = 6.4, 9.2 Hz, 1H), 4.47 (dd, J = 3.2, 8.0 Hz, 1H), 4.17 (dd, J = 2.2, 8.4 Hz, 1H), 3.65 (m, 1H), 3.00 (m, 2H), 2.61 (m, 3H), 2.43 (m, 1H), 2.33 (m, 1H), 2.13 (m, 1H), 2.00 (s, 3H), 1.97−1.74 (m, 9H), 1.58 (m, 1H), 1.40 (d, J = 11.2 Hz, 1H), 1.34 (m, 1H), 1.32 (s, 3H), 1.27 (s, 3H), 0.85 (s, 3H); ^{13}C-NMR (CD_3OD) δ 178.1, 173.7, 173.3, 137.3, 130.5, 129.7, 128.4, 84.6, 77.7, 58.8, 55.4, 55.2, 53.5, 48.1, 41.4, 39.2, 38.2, 37.7, 29.8, 29.6, 28.3, 27.8, 27.5, 25.0, 24.5, 22.2, 15.9 (br), 14.9; IR (CH_2Cl_2) 3270, 3062, 2930, 1644, 1450 cm^{-1}; HRMS calcd for (M + H)$^+$: $C_{30}H_{45}N_4O_5B$ 553.357102, found 553.356124.

Acknowledgment. We would like to thank Dr. Greg Nemeth for NMR work and Dr. Hon-Wah Man for stimulating discussions.

Supporting Information Available: Copies of the ^{1}H NMR and ^{13}C NMR spectra of **6**, **7**, **8**, **10**, **12**, **13**, and **2** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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