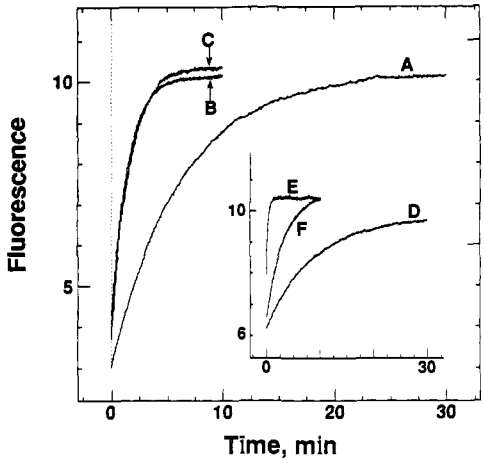
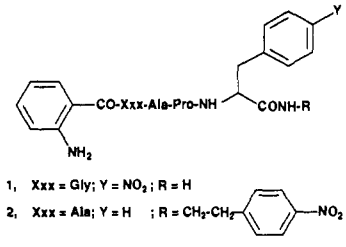


Figure 1. Fluorescent peptidyl prolyl cis-trans isomerization assays using 3.0 μM **1** (curves A–C) and 0.6 μM **2** (curves D–F, inset) at 0.0 ± 0.1 °C: A, uncatalyzed; B, 540 nM human recombinant cyclophilin (hrCyp)[12]; C, 940 nM human recombinant FKBP12 (hrFKBP12).[13] Inset: D, uncatalyzed; E, 11 nM hrCyp; F, 47 nM hrFKBP12. The instrument settings for all assays were identical so that the fluorescent intensities for **1** and **2** could be compared (λ_{ex} = 337 nm, λ_{em} = 410 nm). Assay buffer composition was 25 mM Hepes/NaOH, pH 8.0, 50 mM NaCl; volume of assays was 2.0 mL.

by several analytical methods.[10] The combination of *o*-aminobenzoyl and *p*-nitrobenzyl groups was selected for collisional fluorescence quenching;[7a] the absence of a spectral overlap between the absorption band of the *p*-nitrobenzyl group and the emission spectrum of the *o*-aminobenzoyl group precluded quenching by resonance energy transfer.[11]



Isomerization of the above substrates was followed by monitoring the increase in fluorescence at 410 nm upon excitation at 337 nm. Substrate **1** or **2** was dissolved in a 470 mM solution of LiCl in TFE, which increased the population of the cis isomer to 50–70% as determined by NMR spectroscopy. The increased initial cis substrate concentration produced a 1.7–2.5-fold enhancement in fluorescence upon isomerization to 90% trans (Figure 1). Assays were conducted at 0 °C to minimize the uncatalyzed thermal isomerization (Figure 1). At this temperature, the rate constant k_c for the uncatalyzed cis to trans isomerization was 2.2 × 10^{-3} s^{-1} for **1** and 1.2 × 10^{-3} s^{-1} for **2**, with activation free energies of 19.3 and 19.5 kcal/mol, respectively. Under subsaturating conditions, the disappearance of excess cis conformer (concentration [C] at time t) is described by the integrated eq 1 derived in the supplementary material. $[S]_0$ is the total substrate

$$[C] = [C]_0 e^{-(k_c' + k_c + k_t' + k_t)t} + \frac{k_t' + k_t}{k_c' + k_c + k_t' + k_t}[S]_0(1 - e^{-(k_c' + k_c + k_t' + k_t)t}) \quad (1)$$

concentration, $[C]_0$ is the initial cis conformer concentration, k_c and k_t are the thermal rate constants for cis → trans and trans → cis isomerization, and k_c' and k_t' are apparent first-order rate constants defined as $[E]_0 k_{cat}^{(c)}/K_m^{(c)}$ and $[E]_0 k_{cat}^{(t)}/K_m^{(t)}$, respectively. $[E]_0$ is the enzyme concentration; upper indexes (c) and (t) in k_{cat} and K_m refer to cis → trans and trans → cis catalysis, respectively.

The continuous spectrofluorometric direct assay for PPIases can be used to detect new PPIases and new PPIase inhibitors; for example, inhibition of cyclophilin by CsA is easily detected. Analogues of substrates **1** and **2** should facilitate determining the substrate specificity of PPIases, particularly in the C-terminal region which cannot be characterized by reported coupled assays.

Acknowledgment. The provision of a NATO postdoctoral fellowship to C. García-Echeverría is gratefully acknowledged. This work was supported in part by grants from the National Institutes of Health (GM 40092, AR 32007, and AI24650). We thank Dr. T. Holzman (Abbott Research Laboratories) and Dr. Mark Levy (Smith Kline Beecham) for the gifts of recombinant human cyclophilin and hrFKBP12, respectively.

Supplementary Material Available: Derivation of the initial rate equation (eq 1) and the procedure for the synthesis of peptide **1** (6 pages). Ordering information is given on any current masthead page.

(9) Peptide **1** was synthesized by solid-phase techniques using a Fmoc approach and the "PAL" handle (Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hundson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730–3743) for establishing the C-terminal peptide amide. Peptide **2** was synthesized by classical solution methods (Bodanszky, M.; Bodanszky, A. In *Practice of Peptide Chemistry*; Springer-Verlag: New York, 1984) using N^{α}-*tert*-(butyloxycarbonyl)-protected (Boc) amino acids.

(10) Peptides **1** and **2** were characterized by high-resolution fast atom bombardment mass spectrometry (HR-FABS calcd for $C_{26}H_{32}N_7O_7$ (**1**) 554.2363, found 554.2344; $C_{35}H_{42}N_7O_7$ (**2**) 672.3148, found 672.3129) and by HPLC on a Vydac C-18 column (4.6 × 250 mm): linear gradient over 20 min of CH_3CN/0.036% TFA and H_2O/0.045% TFA from 1:19 to 4:1, flow rate 1.2 mL/min, detection at 214 and 254 nm; single peak at t_R = 12.9 min (**1**), t_R = 18.0 min (**2**). The ^1H NMR spectra of **1** and **2** were consistent with the structures.

(11) (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819–846. (b) Fairclough, R. H.; Cantor, C. R. *Methods Enzymol.* **1978**, *48*, 347–379. (c) Förster, T. *Discuss. Faraday Soc.* **1959**, *27*, 7–17.

(12) Holzman, T. F.; Egan, D. A.; Edalji, R.; Simmer, R. L.; Helfrich, R.; Taylor, A.; Burres, N. S. *J. Biol. Chem.* **1991**, *266*, 2474–2479.

(13) Standaert, R. F.; Galat, A.; Verdine, G. L.; Schreiber, S. L. *Nature* **1990**, *346*, 671–674.

Keto Boronate Reduction: A Novel Method for High 1,3-Relative Asymmetric Induction†

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Received December 5, 1991

Although the methods for establishing 1,3-asymmetric induction in systems possessing heteroatoms at both stereocenters of interest are legion, there exist but a handful of approaches to 1,3-asymmetric induction in compounds where a heteroatom does not comprise one of the substituents of the stereodirecting center.[3] A general method for accomplishing this type of transformation is described herein.

Recent investigations in our laboratories have employed novel intramolecular conformational control elements to achieve high relative asymmetric induction in carbonyl addition reactions.[4] In the present study, 2-alkyl-4-keto 1-boronate esters are showcased as substrates wherein both conformational control and facial

† Dedicated with great warmth and respect to Professor Herbert C. Brown on the occasion of his 80th birthday.

(1) Alfred P. Sloan Foundation Fellow, 1987–1991.

(2) National Institutes of Health Postdoctoral Fellow, 1991–1993.

(3) (a) Evans, D. A.; Bartroli, J.; Godel, T. *Tetrahedron Lett.* **1982**, *23*, 4577. (b) Askin, D.; Volante, R. P.; Ryan, K. M.; Reamer, R. A.; Shinkai, I. *Tetrahedron Lett.* **1988**, *29*, 4245. (c) Sturm, T.; Marolewski, A. E.; Rezenka, D. S.; Taylor, S. K. *J. Org. Chem.* **1989**, *54*, 2039. (d) Koreeda, M.; Hamann, L. G. *J. Am. Chem. Soc.* **1990**, *112*, 8175. (e) Hanessian, S.; Di Fabio, R.; Marcoux, J.-F. Prud'homme, M. *J. Org. Chem.* **1990**, *55*, 3436.

(4) (a) Molander, G. A.; Haar, J. P., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 3608. (b) Molander, G. A.; Cameron, K. O. *J. Org. Chem.* **1991**, *56*, 2617. (c) Molander, G. A.; Etter, J. B.; Harring, L. S.; Thorel, P.-J. *J. Am. Chem. Soc.* **1991**, *113*, 8036.

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Scheme I

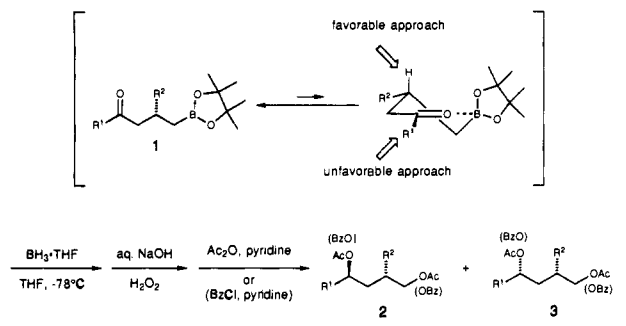

Table I. Results of the Diastereoselective BH_3·THF Reduction of Keto Boronates **1a–h**

substrate	R^1	R^2	% isltd yield (2 + 3)[a]	diastereoselectivity (2:3)[b]
1a	Me	Me	71	34:1[c]
1b	Me	Et	84	22:1[d]
1c	Me	*i*-Pr	89	62:1
1d	Me	Ph	88[e]	24:1
1e	Et	Me	91	28:1[d]
1f	*i*-Pr	Me	92	>50:1[d]
1g	Ph	Me	86	20:1
1h	Pr	Me	88[f]	19:1

[a] Refers to yields of purified material. All of these compounds have been fully characterized spectroscopically (^1H NMR, ^{13}C NMR, IR), and elemental composition has been established by combustion analysis and/or high-resolution mass spectrometry. [b] Determined by fused silica capillary GC analysis on the crude diacetate unless otherwise indicated. [c] Analyzed as the dibenzoate ester. [d] Ratio estimated from ^{13}C NMR data. [e] Reduction performed at −40 °C. [f] Isolated as the diol.

selectivity in carbonyl addition can be achieved via intramolecular complexation of the alkylboronate ester Lewis acid with the carbonyl oxygen. Keto boronate starting materials necessary for our investigation were readily synthesized by the elegant procedure of Knochel.[5] By analogy to processes developed by Narasaka,[6] Prasad,[7] and others[8] in which highly selective 1,3-asymmetric induction via boron or other metal complexes has been utilized to provide convenient routes to 1,3-diols, we envisioned that high diastereoselectivities might arise upon reduction of the keto boronate substrates as a result of stereoelectronically preferred[9] axial attack of a nucleophile on a rigid, boron-complexed substrate (Scheme I). In fact, this analysis proved valid as depicted in Table I, in which the isolated yields and diastereomeric ratios of reaction products derived from reduction of **1** with BH_3·THF are displayed.[10] Although several different reductants [e.g., $NaBH_4$, BH_3·SMe_2, L-Selectride, LS-Selectride, $NaBH_3CN$, $Na(AcO)_3BH$] were tested in these reactions, BH_3·THF provided the best results with consistently high diastereoselectivities.[11] That the anti isomer predominates was established by spectral comparison of the major diol prepared from **1a** with literature data reported for the same compound[12] and by independent synthesis.[13] The stereochemistry of the remainder of the compounds was assigned by analogy.

Although we postulate that a boron-complexed intermediate is responsible for the observed diastereoselectivity, we have no *direct* evidence that such a species exists. Attempts to detect such an intermediate by low-temperature NMR (^1H, ^{13}C, and ^{11}B) and ambient-temperature solution IR spectroscopy were unfruitful, a result not surprising in light of an unsuccessful search for similar intermediates by Prasad and co-workers.[7c,14] It is clear that under the reaction conditions no disproportionation between BH_3·THF and the boronate is occurring. Thus, treatment of pinacol *n*-butylboronate with BH_3·THF even at ambient temperatures for several hours shows no ligand exchange as evidenced by ^{11}B NMR spectroscopy. The high diastereoselectivities observed in the current study thus imply that, although the equilibrium concentration of a complexed species is extremely low, the reduction may proceed exclusively through this activated complex by an intermolecular hydride delivery.

The synthetic method described in this report represents the first use of a carbon-bound organometallic/ketone intramolecular complex as a conformational tool in diastereoselective carbonyl addition reactions. These substrates are attractive because the organometallic moiety can subsequently be converted to more highly functionalized end products by a multitude of diverse reactions. In the present case, for example, the boronate ester products can conceivably be further transformed in situ by oxidation, protonation, Suzuki-type coupling,[15] Matteson homologation,[16] and numerous other processes.[16,17] In addition, analogous substrates can be designed for examination of 1,2 and 1,4 relative asymmetric induction, and high enantiomeric bias may even be possible using chiral boronate esters.[18] The various structural features and potentially useful transformations afforded by these systems thus provide exceptional versatility in the ultimate conversion to a variety of stereodefined organic compounds. Studies in these and related areas are continuing.

Acknowledgment. We gratefully acknowledge the National Science Foundation and the National Institutes of Health for their generous support of our program. In addition, we would like to thank Professor Paul Knochel for helpful comments on the synthesis of keto boronates utilized in this study.

Supplementary Material Available: Complete experimental details and spectral data for synthesis of compounds **1a–g** and **2a–g** (**3a–g**) and details of the structure proof outlined within the text (8 pages). Ordering information is given on any current masthead page.

(5) (a) Knochel, P. *J. Am. Chem. Soc.* **1990**, *112*, 7431. (b) Wuts, P. G. M.; Thompson, P. A. *J. Organomet. Chem.* **1982**, *234*, 137.
(6) Narasaka, K.; Pai, F. C. *Tetrahedron* **1984**, *40*, 2233.
(7) (a) Kathawala, F. G.; Prager, B.; Prasad, K.; Repic, O.; Shapiro, M. J.; Stabler, R. S.; Widler, L. *Helv. Chim. Acta* **1986**, *69*, 803. (b) Chen, K. M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155. (c) Chen, K. M.; Gunderson, K. G.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Chem. Lett.* **1987**, 1923.
(8) For pertinent references, see: Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560.
(9) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983.
(10) Reductions were performed at −78 °C in THF (approximately 1 mmol scale reactions, [**1**] = 0.20 M, 2 equiv of BH_3·THF). Reaction times were 3–4 h, except for **1d** which required a reaction time of −40 °C (dry ice/CH_3CN) for reasonable reaction times. The reduced boronate was oxidized in situ with 10 equiv of a 2 M $NaOH/H_2O$ solution followed by 10 equiv of a 30% H_2O_2 solution. The resulting mixture was extracted with Et_2O, and the aqueous layer was extracted five times with ca. 10 mL of Et_2O. The combined organic layers were dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed in vacuo to yield an oil which was esterified by standard procedures without further purification.
(11) For example, reaction of $NaBH_4$ with substrate **1e** provided a 19:1 mixture of syn/anti diastereomers in 78% isolated yield.
(12) Chiarello, J.; Joullié, M. M. *Synth. Commun.* **1989**, *19*, 3379.
(13) Alkylation of γ-valeroactone leads to a 5:1 mixture of trans/cis 2-methyl-γ-valerolactones. Reduction with $LiAlH_4$ provides the corresponding diols, which can be esterified to provide the benzoates **2a** and **3a**. Hanessian, S.; Murray, P. J.; Sahoo, S. P. *Tetrahedron Lett.* **1985**, *26*, 5623 and references therein. Furthermore, an authentic sample of (2*R**,4*R**)-2-methylheptane-1,4-diol has been prepared by hydrogenation of (2*R**,4*R**)-2-methyl-1,4-bis(benzyloxy)-6-heptene. The latter was obtained by a modification of the procedure described in ref 4a. The diol was shown to be diastereomeric to the compound prepared from **1h** by the method described herein.
(14) A crystal structure depicting intramolecular complexation between an amide carbonyl oxygen and a weakly Lewis acidic boronate ester ($pK_a \approx 10$) has been reported. Matteson, D. S.; Michnick, T. J.; Willett, R. D.; Patterson, C. D. *Organometallics* **1989**, *8*, 726. We thank a referee for bringing this to our attention.
(15) Sato, M.; Miyaura, N.; Suzuki, A. *Chem. Lett.* **1989**, 1405.
(16) Matteson, D. S. *Tetrahedron* **1989**, *45*, 1859.
(17) Brown, H. C.; Singaram, B. *Acc. Chem. Res.* **1988**, *21*, 287.
(18) (a) Roush, W. R.; Walts, A. E.; Hoong, L. K. *J. Am. Chem. Soc.* **1985**, *107*, 8186. (b) Roush, W. R.; Halterman, R. L. *J. Am. Chem. Soc.* **1986**, *108*, 294. (c) Roush, W. R.; Hoong, L. K.; Palmer, M. A. J.; Park, J. C. *J. Org. Chem.* **1990**, *55*, 4109. (d) Roush, W. R.; Hoong, L. K.; Palmer, M. A. J.; Straub, J. A.; Palkowitz, A. D. *J. Org. Chem.* **1990**, *55*, 4117. (e) Roush, W. R.; Ando, K.; Powers, D. B.; Palkowitz, A. D.; Halterman, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 6339.