Bisubstrate Reaction Templates. Examination of the Consequences of Identical versus Different Binding Sites

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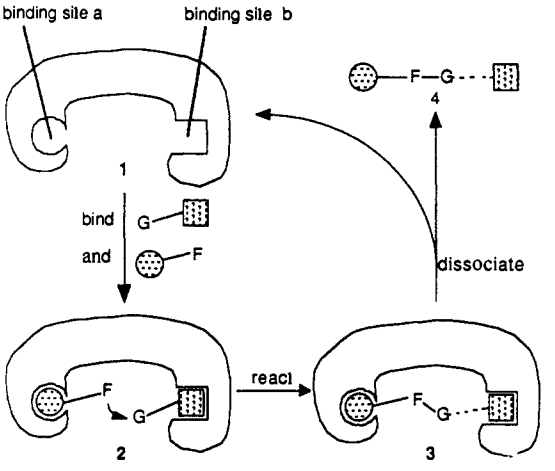
Abstract: A reaction template (**1≈9**) possessing two binding sites is described. The template was designed to use hydrogen bonding to simultaneously (but transiently) bind two substrates, giving rise to a ternary complex (**2≈12**), which positions the substrates in an orientation that facilitates reaction between them. Bisubstrate reaction template **9** was synthesized and shown to accelerate the reaction between substrates **10** and **11**. Control studies support the proposed intermediacy of ternary complex **12**. A second template (**41**), where—in contrast to **9**—the two binding sites are different, is also described. In accordance with prediction, **41** is more effective than **9** at promoting the reaction of its substrates. The reaction promoted by **41** was also shown to be subject to competitive inhibition.

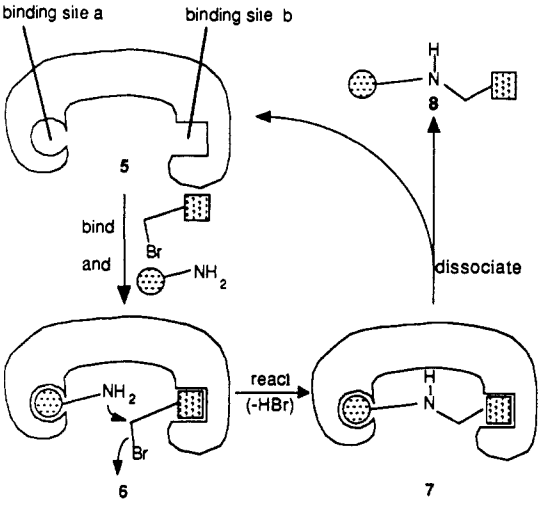
The ability to construct "artificial" enzymes for which there are no natural counterparts would make possible innumerable reactions beyond the reach of current methodology. To date, studies in the area of artificial enzymes[1] have focused almost exclusively on systems where bond cleavage is the dominant theme; the serine protease mimics of Cram[2] and Breslow[3] are conspicuous examples.

From the standpoint of a synthetic chemist, however, the development of systems that facilitate *bond formation* rather than *bond cleavage* is perhaps of greater utility. We envisioned bisubstrate reaction templates to operate as generalized in Scheme I. Thus, the reaction template (**1**) would temporarily—but simultaneously—bind the two substrates (→ **2**), thereby placing the relevant functional groups (F and G) of the two substrates in a position conducive for reaction to occur between them. As a consequence, the reaction between the two substrates becomes effectively intramolecular and reaps the kinetic advantages of intramolecularity.[4,5] Dissociation[8] of the resulting enzyme-product(s) complex **3** furnishes product (**4**) and frees the reaction template (**1**) for another cycle.[9]

Scheme I



Scheme II



(1) (a) For a review, see: Tabushi, I. *Tetrahedron* **1984**, *40*, 269-292. Among more recent *leading* references to this rapidly growing field, see: (b) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89-112. (c) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009-1020. (d) Breslow, R. *Adv. Enzymol.* **1986**, *58*, 1-60. (e) Breslow, R. *Chemtracts: Org. Chem.* **1988**, *1*, 333-348. (f) Rebek, J., Jr. *Science (Washington, D.C.)* **1987**, *235*, 1478-1484. (g) Rebek, J., Jr. *Chemtracts: Org. Chem.* **1989**, *2*, 337-352. (h) Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 362-386. (i) Menger, F. M.; Whitesell, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 707-708. (j) Sasaki, S.; Shionoya, M.; Koga, K. *J. Am. Chem. Soc.* **1985**, *107*, 3371-3372. (k) Klotz, I. M. In *Enzyme Mechanisms*; Page, M. I., Williams, A., Eds.; Royal Society of Chemistry: London, 1987; pp 14-34. (l) Stoddart, J. F. in ref 1k, pp 35-55. (m) Bender, M. L. in ref 1k, pp 56-66. (n) Kirby, A. J. in ref 1k, pp 67-77. (o) Corey, E. J. *Chem. Soc. Rev.* **1988**, *17*, 111-133. (p) Note also: Menger, F. M.; Ladika, M. *J. Am. Chem. Soc.* **1987**, *109*, 3145-3146. (q) Hahn, K. W.; Klis, W. A.; Stewart, J. M. *Science (Washington, D.C.)* **1990**, *248*, 1544-1547. (r) A number of other highly relevant papers, which were presented at the International Symposium of Bioorganic Chemistry (New York, May 1985) are assembled in: *Ann. N.Y. Acad. Sci.* **1986**, *471*, 1-325. (s) For some possible long-term applications, see: Drexler, K. E. *Engines of Creation*; Anchor Press/Doubleday: Garden City, NY, 1986.

(2) Cram, D. J.; Katz, H. E. *J. Am. Chem. Soc.* **1983**, *105*, 135-137. Cram, D. J.; Lam, P. Y.-S. *Tetrahedron* **1986**, *42*, 1607-1615.

(3) Trainor, G. L.; Breslow, R. *J. Am. Chem. Soc.* **1981**, *103*, 154-158. Breslow, R.; Trainor, G. L.; Veno, A. *J. Am. Chem. Soc.* **1983**, *105*, 2739-44.

(4) (a) Page, M. I. *Chem. Soc. Rev.* **1973**, *2*, 295-323. (b) Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219-410. (c) Czarnik, A. W. In *Mechanistic Principles of Enzyme Activity*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers, Inc.: New York, 1988; pp 75-117.

(5) Pauling's proposal[6] that, in addition to rendering reactions effectively intramolecular, enzymes also selectively stabilize transition states is widely—but not universally[7]—accepted. (a) For a recent discussion, see: Kraut, J. *Science (Washington, D.C.)* **1988**, *242*, 553-540. See also: (b) Jencks, W. P. *Cold Spring Harbor Symp. Quant. Biol.* **1987**, *52*, 65-73. (c) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1985.

(6) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375. See also: Haldane, J. B. S. *Enzymes*; Longmans, Green and Co.: London, 1930, p 182.

(7) For a recent summary, see: (a) Page, M. I. In ref 1k, pp 1-13. (b) See also: Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128-134.

(8) The dashed lines in **3** and **4** in Scheme I are meant to indicate that, in principle, the recognition element in one of the substrates can be incorporated into a leaving group. Such a design feature could be used to circumvent complications arising from debilitating product inhibition.

(9) Assuming that bond formation is the rate-limiting step, one can imagine ultimately incorporating additional features into **1**/**2** that would selectively stabilize the transition state[5] and produce further rate enhancement.

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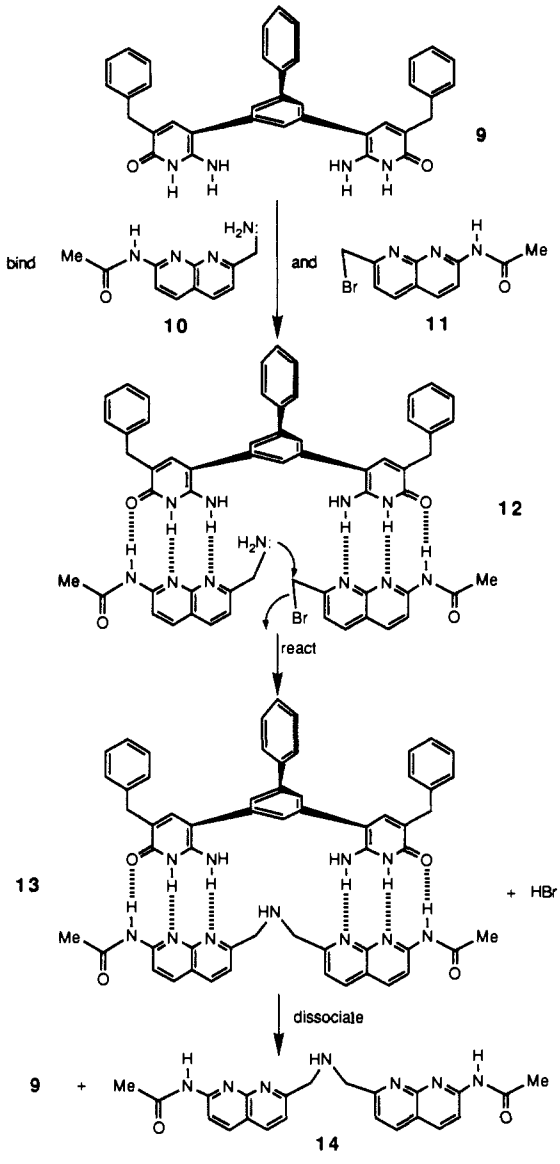
Scheme III

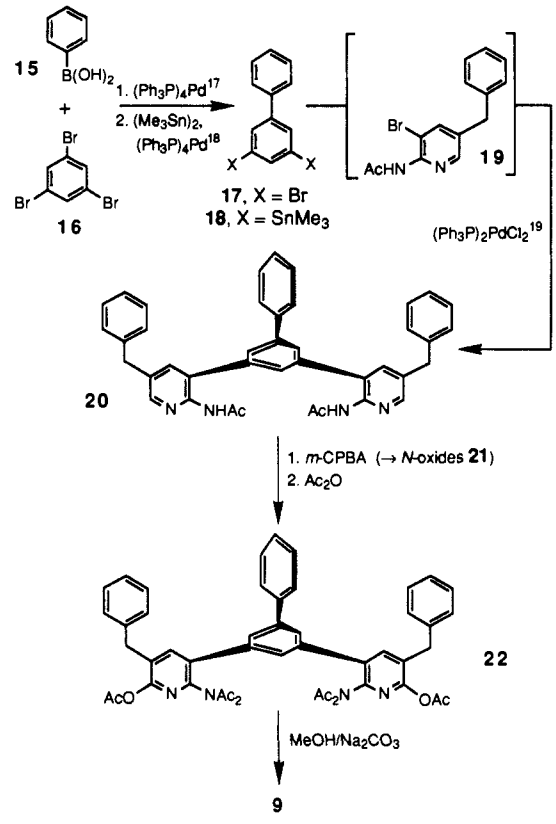


We now report in full detail[10] the first[11,12] example of a designed,[13] fully synthetic[14] system, which, by virtue of a transient ternary complex (as in **2**), accelerates what would otherwise be an intermolecular reaction.[4] We also describe the previously unreported results of an initial step aimed toward understanding how the effectiveness of such systems can be enhanced. It is the hope that these and future studies will help to elucidate some of the design criteria necessary to the eventual practical[15] application of bisubstrate reaction templates in synthesis.

For purposes of simplicity, the mechanistically straightforward S_N2 alkylation of an amine by an alkyl halide (Scheme II) was selected for initial study. Scheme III provides molecular detail, where **10** and **11** are the substrates, **9** is the template, and **12** is the ternary complex. The specifics of **9**–**12** were designed by using CPK models, taking into account synthetic accessibility, and with provision made for solubility in nonpolar organic solvents,[15] whose use was intended to foster hydrogen bonding as the basis for recognition and binding between template and substrates.[16]

The synthesis of **9**, which is summarized in Scheme IV, takes advantage of several recent[17-19] advances in organopalladium chemistry.[20] Thus, three successive palladium-catalyzed couplings serve to assemble the skeleton (**20**) of the template. Bis *N*-oxide[21]

Scheme IV



(10) For a preliminary communication of the system outlined in Scheme III, see: Kelly, T. R.; Zhao, C.; Bridger, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 3744–3745.

(11) (a) An aza-crown ether that sequentially (rather than simultaneously) operates on two substrates (by a "ping-pong"[11b] mechanism) has been reported by Lehn and colleagues: Lehn, J.-M. *Ann. N.Y. Acad. Sci.* **1986**, *471*, 41–50, and references therein. See also: Jahansouz, H.; Jiang, Z.; Himes, R. H.; Mertes, M. P.; Mertes, K. B. *J. Am. Chem. Soc.* **1989**, *111*, 1409–1413. (b) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: New York, 1979; pp 220–222. See also: Reference 5c, pp 115–119, and references therein.

(12) For a very recent report on an ingenious system that also involves a ternary complex, see: Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249–1250.

(13) For examples of less structurally defined complexes that promote reactions between organic substrates, see: Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075–1077. Tabushi, I.; Fujita, K.; Kawakubo, H. *J. Am. Chem. Soc.* **1977**, *99*, 6456–6457. Rideout, D. C.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *102*, 7816–7817. Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. *J. Org. Chem.* **1983**, *48*, 3619–3620. Schneider, H.-J.; Sangwan, N. K. *J. Chem. Soc., Chem. Commun.* **1986**, 1787–1789. Schneider, H.-J.; Sangwan, N. K. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 896–897. Diederich, F.; Lutter, H.-D. *J. Am. Chem. Soc.* **1989**, *111*, 8438–8446. See also: Duerr, B. F.; Czarnik, A. W. *Tetrahedron Lett.* **1989**, *30*, 6951–6954, and references therein.

(14) Some catalytic antibodies also operate via ternary complexes: Schultz, P. G. *Acc. Chem. Res.* **1989**, *22*, 287–294. Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1283–1295. Lerner, R. A.; Benkovic, S. J. *Chemtracts: Org. Chem.* **1990**, *3*, 1–36.

(15) The benzyl groups in **9** are included for solubility; compounds similar to **9** but lacking the benzyl groups were not sufficiently soluble in solvents such as $CDCl_3$ to be useful in the present studies.

(16) For earlier studies of receptor–substrate binding from this laboratory, see: Kelly, T. R.; Maguire, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 6549–6551. Kelly, T. R.; Bilodeau, M. T.; Bridger, G. J.; Zhao, C. *Tetrahedron Lett.* **1989**, *30*, 2485–2488.

(17) (a) Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*, 513–519. (b) Sharp, M. J.; Snieckus, V. *Tetrahedron Lett.* **1985**, *26*, 5997–6000.

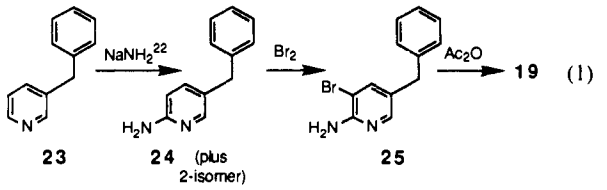
(18) Azizian, H.; Eaborn, C.; Pidcock, A. *J. Organomet. Chem.* **1981**, *215*, 49–58.

(19) (a) Bailey, T. R. *Tetrahedron Lett.* **1986**, *27*, 4407–4410. (b) Kosugi, M.; Koshiba, M.; Atoh, A.; Sano, H.; Migita, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 677–679. (c) Malstein, D.; Stille, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 4992–4998.

(20) It perhaps warrants noting as a commentary on the rapid pace of advances in organic synthesis that even a dozen years ago, when palladium-based biaryl couplings[17-19] were much less developed, the fabrication of **9** would have been a task of substantially more daunting dimension.

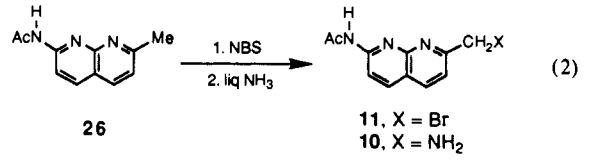
(21) For reviews of pyridine *N*-oxides, see: Shaw, E. N. In *Pyridine and its Derivatives Part Two*; Klingsberg, E., Ed.; Interscience: New York, 1961; pp 97–153. Abramovitch, R. A.; Smith, E. M. In *Pyridine and its Derivatives Supplement Part Two*; Abramovitch, R. A., Ed.; Wiley: New York, 1974; pp 1–261.

formation (→ **21**), *N*-oxide rearrangement (→ **22**), and methanolysis then furnish template **9**. The precursor (**19**) to the binding sites was prepared as indicated in eq 1 (note that the amino group

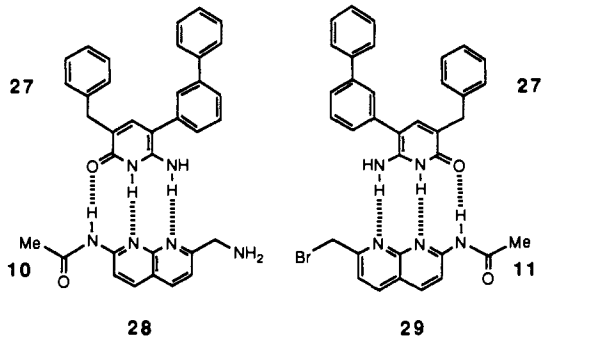


in **24** not only ultimately serves as a hydrogen-bonding site, but also directs bromination into the pyridine ring rather than the otherwise more reactive phenyl ring).

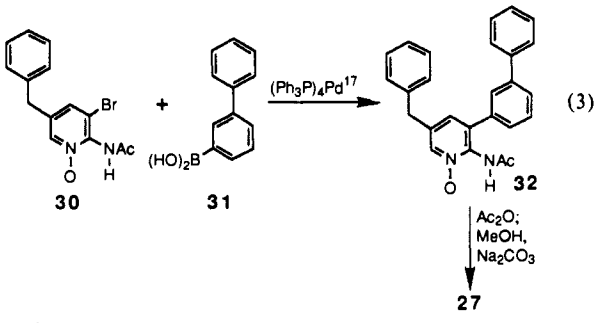
The two substrates, **10** and **11**, were synthesized by the sequence outlined in eq 2. The affinities (association constants, K_{assoc}'s)



of the substrates for the binding sites in **9** were estimated by using **27** (prepared as summarized in eq 3) as the receptor; K_{assoc}'s



($CDCl_3$) for **28** (**27·10**) and **29** (**27·11**) are 1.2×10^4 (±10%) and 1.7×10^4 (±10%) M^{-1}, respectively.



Kinetic experiments demonstrate that **9** promotes the reaction between **10** and **11** and are in agreement with **9** functioning (Scheme III) as a bisubstrate reaction template. Figure 1 illustrates the difference in the rate of reaction of **10** with **11** (both 0.0040 M) in the presence and in the absence of **9**. Calculation of the initial rates from the data in Figure 1 reveals that inclusion of 1 equiv of **9** produces a 6-fold rate increase.[23] That the rate

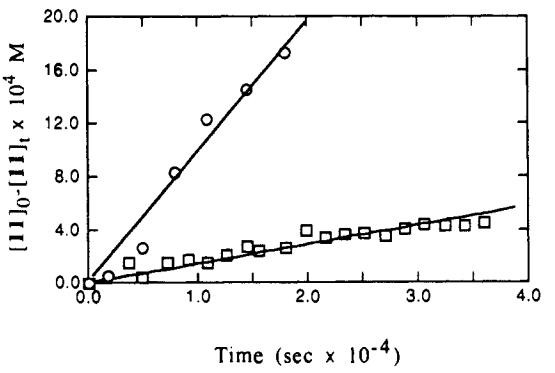


Figure 1. Plot of the early stages of the reactions of **10** and **11** (0.0040 M each) in the absence (□) and presence (○, 0.0040 M) of **9** in $CDCl_3$ at 25 °C. The concentration of **11** was monitored by ^1H NMR, integrating against an internal standard (see Experimental Section for details). Slopes of the straight lines are the approximate initial rates.

acceleration results from the transient intermediacy of ternary complex **12** is supported by (i) ^1H NMR titration studies[24] using **9** and **26**, which establish that **9** is capable of simultaneously binding 2 equiv of **26**, and (ii) the association constants (above) for **27·10** and **27·11**, which indicate that under the conditions of reaction **9** exists primarily as ternary complex. Control experiments confirm that a template with two binding sites is required for rate acceleration: addition of 1 or 2 equiv of **27** to a solution 0.0040 M in both **10** and **11** has no effect on their rate of intermolecular reaction.

Owing to the relatively modest rate acceleration observed (and the competing[23] uncatalyzed reaction between amine **10** and bromide **11**), it was not possible to unequivocally prove (or disprove) that turnover was occurring,[25] although we believe that **9** is capable of turnover. That belief is based on two observations. First, for *all* the binding interactions between host/guest partners that are described in this paper, exchange is rapid on the NMR time scale, which is equivalent to an on/off rate of greater than $\sim 1 \times 10^3$ s^{-1} at 25 °C.[26] That exchange is rapid is clear from examination of the ^1H NMR spectra of non-1:1 ratios of host and guest: only average spectra are observed (rather than superimposed spectra of bound and unbound species). The second observation consistent with a capability for turnover is that the product (**14**) precipitates from the reaction solution as its (unbound) hydrobromide salt. In the present case, the precipitation of **14**·HBr prevents what might otherwise be serious[8] product inhibition, since the product can be bound to the template by twice as many hydrogen bonds as an individual substrate.

One can ask whether the substitution of a less reactive substrate for **11** in Scheme III will have an effect on the ratio of the catalyzed-to-uncatalyzed reaction rates since, upon first reflection, it might appear that **9** would be more effective at catalyzing a reaction involving a less reactive substrate. To that end, chloride **33** was substituted for bromide **11**. In the absence of **9**, the rate of the intermolecular reaction of **10** with **33** is approximately 70-fold slower[27] than the uncatalyzed reaction of **10** with **11**. In

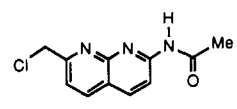
(22) Robison, M. M.; Robison, B. L. *J. Am. Chem. Soc.* **1955**, *77*, 457–460.

(23) A 5-fold increase in the concentrations (to 0.020 M) of both **10** and **11** led to a 24.9-fold rate increase (theory for S_N2, 25×) in the absence of **9**. A 5-fold increase in the concentration of **10** and **11** (to 0.020 M) in the presence of **9** (concentration also increased to 0.020 M) resulted in a rate enhancement of only 16× (rather than 25×), which is consistent with intervention of **12**. [One might predict only a 5-fold increase but, at higher concentrations, a somewhat (note the K_{assoc}'s for **28** and **29**) larger fraction of **10** and **11** is in the form of ternary complex **12**. Probably more importantly, due to the identity of the two binding sites in **9**, two "nonproductive", ternary complexes (**9·10·10** and **9·11·11**) whose concentrations are similar to that of **12** (=**9·10·11**) are also present (see later in text for further discussion); reaction *between* (as opposed to *within*) ternary complexes to give **14** will exhibit a second-order response to an increase in concentration.].

(24) A 0.020 M suspension of **9** in $CDCl_3$ required 2 equiv of **26** to give a homogeneous solution. The chemical shift of the AcN*H* proton of **26** (in the absence of **9**) is somewhat concentration dependent: δ's are 8.63, 8.82, 8.96, and 9.20 ppm when [**26**] = 0.020, 0.040, 0.060, and 0.080 M. For 2:1, 3:1, and 4:1 ratios of **26** to **9** (always 0.020 M in **9**), the δ's are 12.41, 11.58, and 10.74 ppm, respectively (exchange is rapid).

(25) Because the rates of intra- and intermolecular reactions respond differently to dilution, it might be expected that at concentrations lower than 0.0040 M one would be better able to determine whether turnover is occurring. Unfortunately, the binding constants are such that, at concentrations sufficiently lower to shed potential light, the fraction of **9** existing as ternary complex is considerably reduced. Also, under such more dilute conditions, signal-to-noise constraints (^1H NMR) diminish the accuracy of the data (since a time-dependent process is being monitored, merely increasing the number of ^1H NMR acquisitions does not offer a simple way around the problem). Consequently, attempts to employ more dilute samples to probe the question of turnover were not fruitful.

(26) Pople, J. A.; Schneider, W. G.; Bernstein, H. J. *High Resolution Nuclear Magnetic Resonance*; McGraw-Hill: New York, 1959; Chapter 10.



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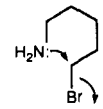

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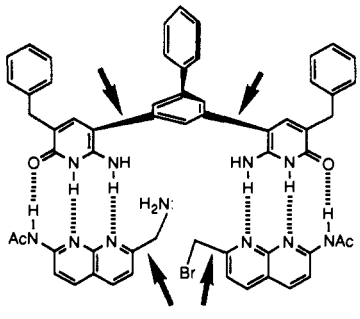
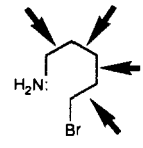
the presence of an equimolar amount of **9**, a rate acceleration of 6—relative to the uncatalyzed reaction of **10** with **33**—is still observed. The same template-induced enhancement in the relative rate of reaction of **10** + **33** (compared with **10** + **11**) is perhaps most easily understood by recognizing that when closely similar *pairs* of intra- and intermolecular reactions are compared, whether the substrates react in bound or unbound form, the same bonds are being broken and formed in both the bound and unbound cases.

The results described above validate the basic concept underlying the design of a bisubstrate reaction template; nonetheless, the absolute magnitude (6×) of the rate enhancement is less than might have been expected. As noted previously, involvement of ternary complex **12** in effect renders the alkylation of **10** with **11** an intramolecular reaction. In contrast to the 6-fold rate increase achieved via ternary complex **12**, the corresponding intramolecular cyclization of amino bromide **34** proceeds approximately 5×10^3 times[28] faster than the intermolecular reaction between triethylamine and ethyl bromide. Both **12** (see arrows in **35**) and



35 (= 12)



36 (= 34)

34 (see arrows in **36**) have the same number (four) of single bonds around which free rotation (to give nonproductive conformers) can occur. Consequently one might have anticipated a more substantial rate enhancement brought about by involvement of ternary complex **12** than the relatively modest enhancement actually observed.

How does one begin to improve the design underlying Scheme III so as to enhance the effectiveness of the template? The simplest first step is to make the two binding sites in the template nonequivalent. Identical binding sites were originally incorporated in **9** because that simplified the synthetic component of the project. The price exacted by such a simplification, however, is that **12** (=**9**·**10**·**11**) is unlikely to be the only ternary complex present in the reaction solution: nonproductive ternary complexes (i.e., **9**·**10**·**10** and **9**·**11**·**11**), where two molecules of the same substrate are bound to **9**, should also be present and will diminish the efficiency of the system.

The binding sites for template **9** consist of linear triads of hydrogen bond donors and acceptors. Because only two possibilities (donor or acceptor) exist for an individual hydrogen-bonding group, linear hydrogen bond triads can be conceptualized as three-digit binary numbers. Eight (2^3) such triads exist. Those

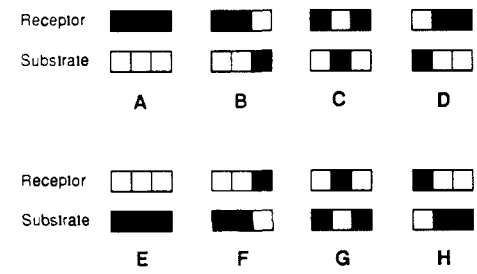
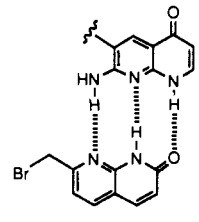


Figure 2. Schematic representation of the eight possible linear arrangements of a triad of hydrogen bonds involving receptors and substrates. Shaded boxes represent hydrogen bond donors; white boxes represent hydrogen bond acceptors.

eight, along with the complementary substrate triads, are shown schematically in Figure 2, with a shaded box representing a donor and a white box an acceptor; the recognition elements in **9**/**12** correspond to hydrogen-bonding patterns B and D. Although Figure 2 shows eight triad pairings, in practice, if one selects a specific triad arrangement for one binding site, not all seven of the remaining options are suitable for use in the second binding site. Thus, for example, if D represents one binding interaction (it corresponds to the **9**·**10** interaction in ternary complex **12**), then one should avoid employing arrangement H for the second binding site (because the two substrates then have complementary hydrogen-bonding triads and could associate with each other). Furthermore, since D and B are merely mirror images (on a vertical mirror plane), inclusion of D and B in the same system will ordinarily lead to complications from competitive formation of nonproductive ternary complexes, because the substrate in D (after merely rotating 180° around a vertical axis) can bind to the receptor in B. In fact, the nonproductive complexes **9**·**10**·**10** and **9**·**11**·**11** are embodiments of precisely this degeneracy. Similar considerations exclude F.

Consequently, if one seeks to differentiate the two binding interactions in the system while preserving one binding site unchanged (say, as in D, the left-hand triad in **9**), then the only worthwhile patterns for the second binding interaction are A, C, E, and G. The hydrogen-bonding pattern of C was selected for the new binding interaction; a specific embodiment of this receptor–substrate pairing is provided in **37**. Even with C (≈**37**),



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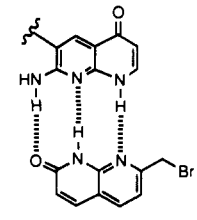

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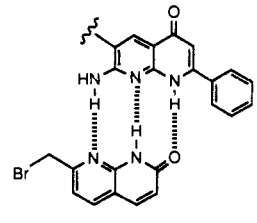
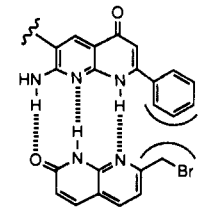


39



40

however, a potential pitfall exists, because C possesses a vertical axis of symmetry with regard to its hydrogen-bonding pattern, and a nonproductive complex (**38**) is also possible. In order to suppress the potential for **38**-like complications, a phenyl substituent was incorporated at C-7 of the receptor (see **39**), since repulsive interactions (see **40**) should thereby attenuate the nonproductive binding.

The new template possessing nonidentical binding sites thus is **41** and the corresponding ternary complex is **43** (Scheme V).

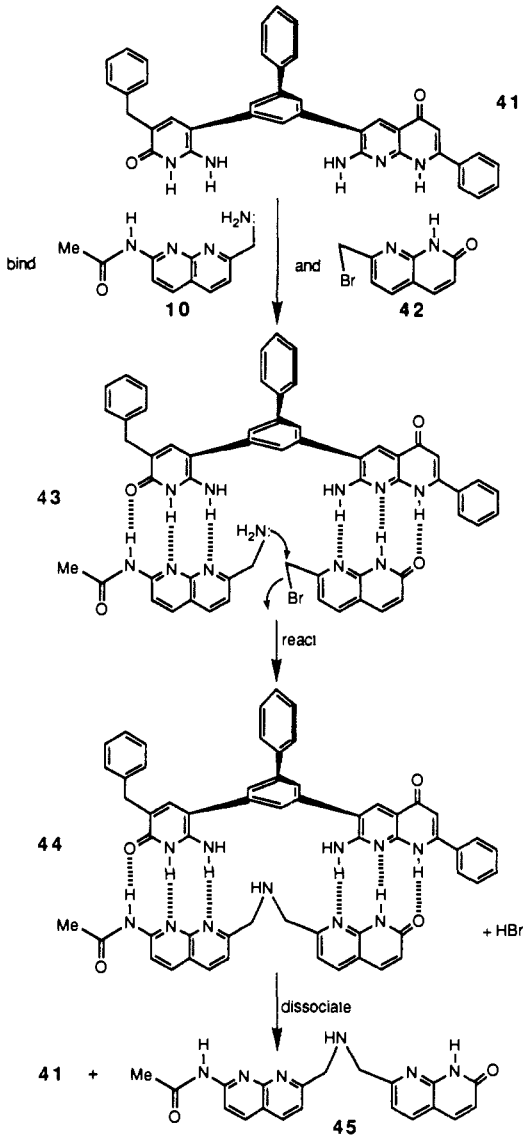
(27) The difference in the rate of reaction between **11** and **33** is typical of the difference in reactivity of benzyl chlorides and benzyl bromides in S_N2 reactions. For a leading reference, see: Streitweiser, A. *Solvolytic Displacement Reactions*; McGraw-Hill: New York, 1962; Chapter III.

(28) Data taken from Table 7 in ref 4a. Strictly speaking, because the comparison in ref 4a is made between the rate constants of a first-order (intramolecular) and second-order (intermolecular) process, the 5×10^3 rate acceleration is designated as "effective molarity".

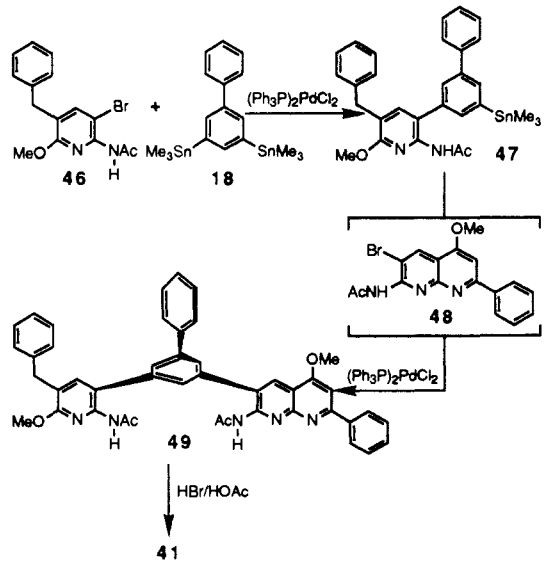
(29) For a related reaction and leading references, see: Kelly, T. R.; Bell, S. H.; Ohashi, N.; Armstrong-Chong, R. J. *J. Am. Chem. Soc.* **1988**, *110*, 6471–6480.

(30) Miller, R. B.; Dugar, S. *Tetrahedron Lett.* **1989**, *30*, 297–300.

Scheme V

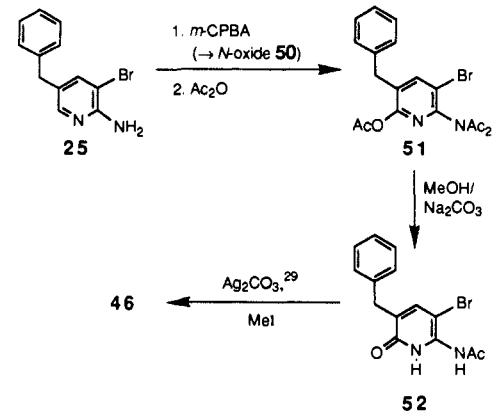


Scheme VI

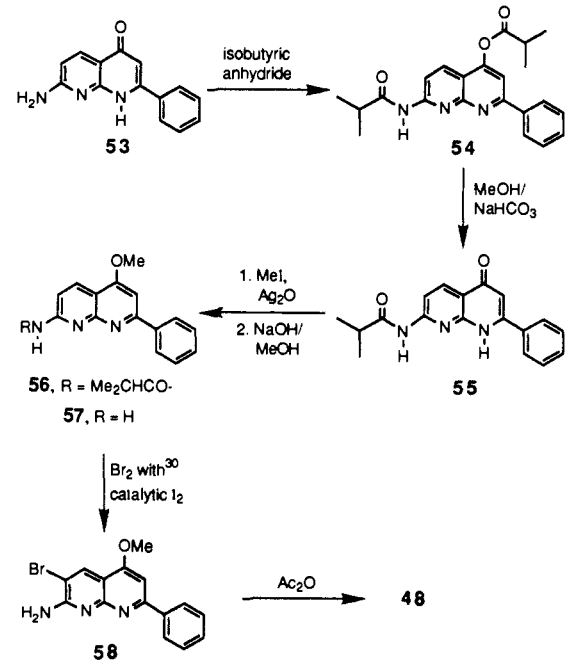


Since **41**, in contrast to **9**, is not symmetrical, the synthesis of **41** proved a more demanding undertaking. The skeleton of template **41** was assembled (Scheme VI) from **46**, **18**, and **48**, again by using palladium-catalyzed couplings for construction of the key biaryl linkages. A single operation then cleaved the four

Scheme VII



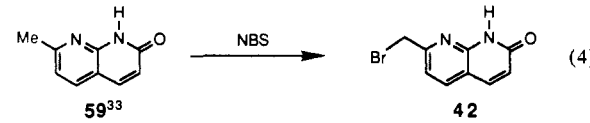
Scheme VIII



protecting groups in **49** and permitted the requisite isomerizations of the two resulting hydroxypyridine units to the pyridone tautomers necessary for the template (**41**). The use of **46** as the synthon for the left-hand binding site is an improvement on our earlier (Scheme IV) synthesis, in that employment of **46** allows for incorporation of the left-hand binding site in a more fully developed form, thereby increasing the convergency of the synthesis. The preparation of **46** is summarized in Scheme VII.

Bromonaphthyridine **48**, the precursor to the right-hand binding site in **41**, was prepared as outlined in Scheme VIII. The sequence of blocking/deblocking steps was necessitated in part by the propensity of **53** and **55** to undergo bromination in the wrong ring (ortho to the oxygen).

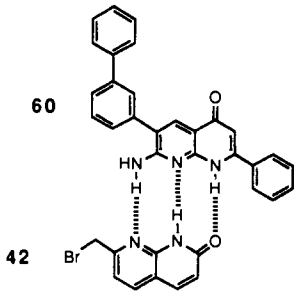
Substrate **10** (Scheme V) was already in hand from the studies above; substrate **42** was prepared as indicated in eq 4. Binding



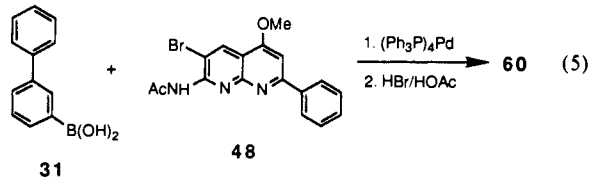
(4)

studies between **60** (see eq 5 for preparation) and **42** indicated that the association constant (K_{assoc}) for complex **61** (=**60**·**42**) is 4.4×10^2 (±15%) M^{-1} in $CDCl_3$; this association constant is a factor of 27 lower than that of the left-hand binding site (**28**).[31]

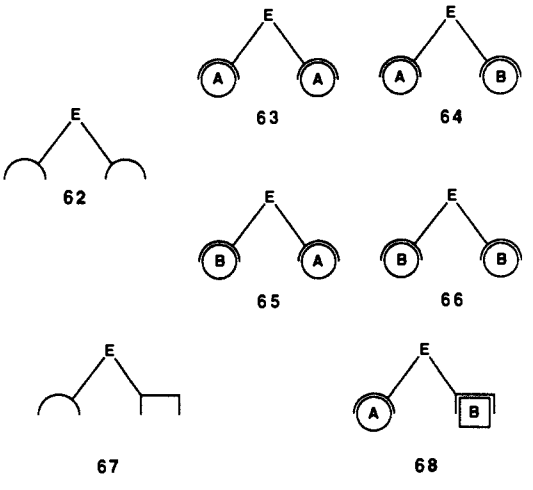
(31) For a possible explanation of the differences between the K_{assoc}'s of **42**·**60** (**61**) and those of **10**·**27** (**28**) and **11**·**27** (**29**), see: Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008–2010.



but strong enough that ternary complex **43** (Scheme V) should still be present in substantial amounts (vide infra) under the reaction conditions.



Presuming that **41**, like **9**, operates via a ternary complex (**43**, Scheme V), one can predict the kinetic advantage conferred by using a system where the binding sites are different rather than identical. If one represents **9**, the template with identical binding sites, in the generic form **62**, then four possible ternary complexes [**63–66** (where A represents amine and B represents bromide)]



can form with equal likelihood (assuming equimolar amounts of A, B, and **62**, and that binding at the two sites is not cooperative). Because of the symmetry of **62** (=**9**), **64** and **65** are equivalent and productive; but **63**, where both binding sites are occupied by amine, and **66**, where both binding sites are occupied by bromide, will be nonproductive. Thus two of the four (**63–66**) possible ternary complexes—i.e., 50%—will be productive. In the case of the template with nonidentical binding sites (represented by **67**), only one ternary complex (**68**) is possible, and it should be productive. Since 50% of the complexes of **62** (=**9**) will be productive and 100% of the complexes of **67** (=**41**) will be productive, one simplistically predicts that **41** will be twice as effective a catalyst as **9**. In the event, under the same conditions (see Figure 1) where **9** gives a 6-fold rate acceleration, use of **41** (Figure 3) results in 12-fold rate enhancement. Control studies are again consistent with the intermediacy of a ternary complex (**43**): addition of 1 equiv of either **27** or **60** (in the absence of **41**) has no effect on the rate of the intermolecular reaction between **10** and **42**.

The agreement between prediction and experiment is impressive, but it is also somewhat illusory. In order to compare Schemes III and V, numerous assumptions must be made. Many of those

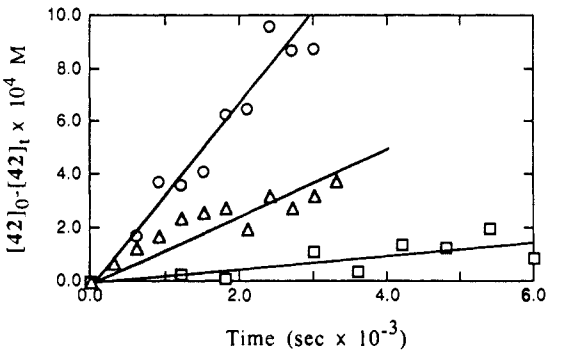


Figure 3. Plot of the early stages of the reactions of **10** and **42** (0.0040 M each) in the absence (□) and presence (○, 0.0040 M) of **41**, and (△) with **26** (0.0040 M) present as an inhibitor of the **41**-catalyzed reaction. The concentration of **42** was monitored by ^{1}H NMR, integrating against an internal standard (see Experimental Section for details). Slopes of the straight lines are the approximate initial rates.

assumptions cancel out (as they did—see above—when chloride **33** was compared with bromide **11** as substrate for **9**), because it is the *relative* rates of reaction of the two systems in the presence and absence of template that are being compared. One can, however, be more precise and use the K_{assoc}'s of **28**, **29**, and **61** to estimate the fraction of ternary complex present in the two systems (Schemes III and V) under the conditions of reaction (~84% in the case of **9**, ~41% in the case of **41**, assuming no allostery in the bindings). By that accounting, it becomes evident that Scheme V is twice as effective as Scheme III, even though only about half as much as **41** (compared to **9**) is actually in the form of ternary complex at any given time. In other words, ternary complex **43** is roughly 4 times as effective as **9**, and **43**'s involvement results in a rate acceleration—compared to the unassisted reaction of **10** with **42**—of approximately 24-fold. The reasons for the additional factor of 2 (24-fold instead of 12-fold) in rate acceleration are not obvious, but it is possible that the geometry, rotamer population, or both of **43** are slightly more conducive than those of **12** to promoting reaction between its substrates. Nonetheless, the qualitative agreement between prediction and experiment provides further support for the conclusion that ternary complexes are central to the overall reaction schemes. That conclusion is additionally buttressed by the finding (Figure 3) that **26** competitively inhibits operation of Scheme V; as expected, inclusion in Scheme V of 1 equiv of **26** reduces the effectiveness of **41** by a factor of approximately 2.

The systems described above are only rudimentary, but the results demonstrate the validity of the design concepts underlying bisubstrate reaction templates. Those results also provide a first example (Scheme V) of how one can rationally proceed to optimize the efficiency of bisubstrate systems. Efforts to further enhance the effectiveness of such assemblies by examining the consequences of increasing (or decreasing) the rigidity of the templates are currently underway. The additional questions of (i) what other reactions are amenable to catalysis by bisubstrate templates and (ii) whether it is possible to incorporate into such systems features that not only properly position reactants but also stabilize[5,9] transition states of ensuing reactions are also under examination.

Experimental Section

General Procedures. Melting points were determined in Pyrex capillaries in a Mel-Temp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer. In all cases, chemical shifts are reported in parts per million (ppm) downfield from internal tetramethylsilane. Routine mass spectra (EI) were obtained by direct insertion using a Hewlett-Packard 5985 GC/MS spectrometer; high-resolution (FAB) mass spectra (see Acknowledgment) were measured on a JEOL HX-110 double-focusing mass spectrometer at a resolution of 10000 using peak matching. Infrared spectra were recorded on a Nicolet 510 FT-IR spectrometer using KBr disks; broad peaks are designated "br". Whatman silica gel PE SIL G/UV plates (250 μm) were used for analytical TLC. For preparative TLC, Analtech silica gel GF plates were employed; after elution, compounds were extracted from the silica gel by using CH_2Cl_2/MeOH (95:5). Flash column

chromatography was conducted according to the procedure of Still et al.[32] with silica gel 60 (average particle size 40 μm, EM Science). Reactions sensitive to air or moisture were conducted in oven- or flame-dried glassware under an atmosphere of dry nitrogen or argon. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl, dichloromethane from calcium hydride. Petroleum ether refers to the fraction boiling from 35 to 50 °C. The phrase "solvent was evaporated" or equivalent phrases mean that solvents were removed on a rotary evaporator at aspirator vacuum and that remaining traces of volatiles were then removed on a vacuum pump. Elemental analyses were performed by Robertson Laboratories Inc., Madison, NJ.

3,5-Bis[5-(6-amino-3-benzyl-2-oxopyridyl)]biphenyl (9). Compound **22** (75 mg, 0.094 mmol) and sodium carbonate (90 mg, 0.73 mmol) in MeOH (10 mL) were stirred overnight at room temperature. The MeOH was evaporated and the residue was partitioned between water and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL), and the combined organic phases were dried (Na_2SO_4) and evaporated to give a brown solid. Purification by preparative TLC developing with CH_2Cl_2/MeOH (9:1) gave **9** (35 mg, 68%) as a pale yellow solid, mp 282–283 °C (dec) after recrystallization from CH_2Cl_2/Et_2O: ^1H NMR (DMSO-d_6) δ 3.60 (4 H, s), 5.73 (4 H, br s), 7.11–7.48 (16 H, m), 7.67–7.70 (4 H, m), 10.70 (2 H, br s); IR (KBr) ν 3472 (br), 3332 (br), 3177 (br), 3022, 2917, 1644, 1616 cm^{-1}; exact mass calcd for $C_{36}H_{31}N_4O_2$ $[M + H]^+$ 551.2447, found 551.2460.

2-(Acetylamino)-7-(aminomethyl)-1,8-naphthyridine (10). Ammonia gas was condensed at −78 °C into a flask (to give ~20 mL of liquid NH_3) containing solid **11** (1.00 g, 3.6 mmol); the suspension was stirred at −78 °C for 2 h and then allowed to warm to room temperature, during which time (~1 h) the excess ammonia evaporated. The white residue was partitioned between chloroform and water. The aqueous layer was separated and extracted with chloroform (4 × 20 mL). The combined organic phases were dried (K_2CO_3) and evaporated to give **10** (418 mg, 54%) as a light purple powder, mp 169–171 °C (dec) after recrystallization from $CHCl_3/Et_2O$: ^1H NMR ($CDCl_3$) δ 2.16 (2 H, br s), 2.27 (3 H, s), 4.20 (2 H, s), 7.35 (1 H, d, J = 8.4 Hz), 8.07 (1 H, d, J = 8.4 Hz), 8.16 (1 H, d, J = 8.8 Hz), 8.48 (1 H, d, J = 8.8 Hz), 8.64 (1 H, br s); IR (KBr) ν 3465 (br), 3332, 3282, 3135, 3064, 3016, 2938, 2832, 1703, 1611 cm^{-1}; mass spectrum, m/e (relative intensity), 216 (46, M^+), 145 (100). An analytical sample of **10** was obtained as its hydrochloride, mp 264–266 °C (dec), after recrystallization from MeOH/Et_2O. Anal. Calcd for $C_{11}H_{13}N_4OCl \cdot {}^1/_2MeOH$: C, 51.40; H, 5.62; N, 20.84. Found: C, 51.04; H, 5.23; N, 20.85.

2-(Acetylamino)-7-(bromomethyl)-1,8-naphthyridine (11). 2-(Acetylamino)-7-methyl-1,8-naphthyridine (**26**; 2.00 g, 9.94 mmol), *N*-bromosuccinimide (Aldrich, 2.00 g, 11.2 mmol), and benzoyl peroxide (Aldrich, 97%, 40 mg) in anhydrous chloroform (Aldrich, stabilized with 0.5–1.0% ethanol, 100 mL) were refluxed with stirring for 6 h while being irradiated with a 250-W Westinghouse household Heat Ray infrared lamp. After being cooled, the reaction mixture was washed with water (5×), dried (Na_2SO_4), and evaporated to give a yellow-brown solid. Purification by flash column chromatography on silica gel (ethyl acetate and then 39:1 CH_2Cl_2/MeOH) gave **11** (733 mg, 26%) as a white solid, which decomposed at 178–180 °C, without melting, to a green solid: mp >300 °C; ^1H NMR ($CDCl_3$) δ 2.23 (3 H, s), 4.64 (2 H, s), 7.55 (1 H, d, J = 8.4 Hz), 8.10 (1 H, d, J = 8.4 Hz), 8.13 (1 H, d, J = 9.0 Hz), 8.48 (1 H, d, J = 9.0 Hz), 8.79 (1 H, br s); IR (KBr) 3184, 3128, 3065, 3029, 2966, 1707, 1609 cm^{-1}; mass spectrum, m/e (relative intensity), 281 (13, M^+), 279 (14, M^+), 158 (100). Anal. Calcd for $C_{11}H_{10}N_3OBr$: C, 47.17; H, 3.60; N, 15.00. Found: C, 47.29; H, 3.52; N, 14.77.

3,5-Dibromobiphenyl (17). A mixture of phenylboronic acid (**15**; 3.30 g, 27.1 mmol), 1,3,5-tribromobenzene (**16**; 10.2 g, 32.4 mmol), tetrakis(triphenylphosphine)palladium(0) (625 mg, 0.541 mmol), 2 M aqueous sodium carbonate solution (27 mL), ethanol (54 mL), and toluene (162 mL) were stirred at 90–95 °C under nitrogen for 8 h. After cooling to room temperature, the organic layer was separated and the aqueous layer was extracted with ether (3 × 50 mL). The combined organic phases were dried ($MgSO_4$) and evaporated to give an oily residue. Purification by flash column chromatography on silica gel, eluting with petroleum ether, afforded **17** as a colorless oil: 5.66 g, 67%; ^1H NMR ($CDCl_3$) δ 7.40–7.63 (m); mass spectrum, m/e (relative intensity), 314 (35, M^+), 312 (70, M^+), 310 (36, M^+), 152 (100).

3,5-Bis(trimethylstannyl)biphenyl (18). 3,5-Dibromobiphenyl (**17**; 2.10 g, 6.7 mmol), hexamethylditin (5.30 g, 16.2 mmol), and tetrakis(triphenylphosphine)palladium(0) (380 mg, 0.30 mmol) in toluene (50 mL) were heated at 110–120 °C for 4 h under argon with stirring. After cooling to room temperature, the solvent was evaporated in vacuo and the residue was purified by flash column chromatography on silica gel, eluting with petroleum ether, to give **18** (2.90 g, 90%) as a white solid: mp 71–73 °C; ^1H NMR ($CDCl_3$) δ 0.34 (18 H, s), 7.34–7.64 (8 H, s); IR (KBr) ν 3451 (br), 3036, 2980, 2910, 1595 cm^{-1}.

2-(Acetylamino)-5-benzyl-3-bromopyridine (19). 2-Amino-5-benzyl-3-bromopyridine (**25**; 4.27 g, 18.3 mmol) was heated in acetic anhydride (60 mL) at 45–50 °C under argon with stirring for 3 h and then stirred at room temperature overnight. The acetic anhydride was removed in vacuo on a Kugelrohr giving an orange-yellow solid residue. Purification by flash column chromatography on silica gel, eluting with 49:1 CH_2Cl_2/MeOH, gave a yellow solid, which was washed with ether to give **19** (3.88 g, 78%) as a white crystalline solid: mp 100–101 °C; ^1H NMR ($CDCl_3$) δ 2.41 (3 H, s), 3.93 (2 H, s), 7.16–7.18 (2 H, m), 7.25–7.35 (3 H, m), 7.65 (1 H, d, J = 1.7 Hz), 7.81 (1 H, br s), 8.21 (1 H, d, J = 1.7 Hz); IR (KBr) ν 3275 (br), 3191 (br), 3057, 3029, 1672 cm^{-1}; mass spectrum m/e (relative intensity) 306 (10, M^+), 304 (11, M^+), 264 (98), 263 (79), 262 (100), 261 (68). Anal. Calcd for $C_{14}H_{13}N_2OBr$: C, 55.10; H, 4.29; N, 9.18. Found: C, 55.09; H, 4.25; N, 8.95.

3,5-Bis[3-[2-(acetylamino)-5-benzylpyridyl]]biphenyl (20). 3,5-Bis(trimethylstannyl)biphenyl (**18**; 1.40 g, 2.9 mmol), 2-(acetylamino)-5-benzyl-3-bromopyridine (**19**; 2.33 g, 7.3 mmol), and bis(triphenylphosphine)palladium(II) chloride (62 mg, 0.09 mmol) in toluene (4.2 mL) were heated at 105–110 °C for 15 h with stirring in a sealed tube under Ar. After cooling, the solvent was evaporated and the residue was purified by flash column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9:1) to give **20** (1.05 g, 60%), as a pale yellow solid: mp 104–105 °C; ^1H NMR ($CDCl_3$) δ 2.16 (6 H, s), 3.99 (4 H, s), 7.19–7.56 (10 H, m), 8.03 (2 H, br s), 8.31 (2 H, d, J = 2.2 Hz); IR (KBr) ν 3388 (br), 3247 (br), 3029, 1679 cm^{-1}; exact mass calcd for $C_{40}H_{31}N_4O_2$ $[M + H]^+$ 603.2760, found 603.2756.

3,5-Bis[3-[2-(acetylamino)-5-benzyl-1-oxopyridyl]]biphenyl (21). To a solution of **20** (1.05 g, 1.74 mmol) in CH_2Cl_2 (50 mL) was added *m*-chloroperoxybenzoic acid (Aldrich, 80%; 1.50 g, 7.0 mmol), and the mixture was stirred overnight at room temperature under argon. The reaction solution was washed with 5% aqueous sodium bicarbonate (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (4 × 25 mL). The combined organic phases were dried ($MgSO_4$) and evaporated. Purification by flash column chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9:1) gave a pale yellow solid. Recrystallization from CH_2Cl_2/ether gave **21** (746 mg, 68%) as a white solid: mp 203–205 °C dec; ^1H NMR ($CDCl_3$) δ 2.00 (6 H, s), 3.92 (4 H, s), 7.17–7.43 (16 H, m), 7.55–7.60 (4 H, m), 8.13 (2 H, s), 9.41 (2 H, br s); IR (KBr) ν 3444, 3064 (br), 2931, 1706, 1589 cm^{-1}. Anal. Calcd for $C_{40}H_{34}N_4O_4$: C, 75.69; H, 5.40; N, 8.82. Found: C, 75.73; H, 5.31; N, 8.60.

3,5-Bis[3-[6-acetoxy-2-(*N*,*N*-diacetylamino)-5-benzylpyridyl]]biphenyl (22). *N*-oxide **21** (300 mg, 0.47 mmol) and acetic anhydride (9 mL) were stirred at 140 °C for 2.5 h under argon (the mixture became dark brown almost immediately). After cooling, excess acetic anhydride was removed in vacuo on a Kugelrohr. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/petroleum ether (1:1), to give **22** (75 mg, 20%) as a pale yellow solid: mp 91–92 °C; ^1H NMR ($CDCl_3$) δ 2.16 (12 H, s), 2.30 (6 H, s), 5.82 (2 H, br s), 6.18 (1 H, s), 6.59 (2 H, br s), 7.08–7.53 (18 H, m), 7.75–7.79 (3 H, m), 7.98 (1 H, s), 10.72 (1 H, br s), 11.66 (1 H, br s); IR (KBr) ν 3742 (br), 3029, 1771, 1721, 1602 cm^{-1}.

2-Amino-5-benzylpyridine (24). 3-Benzylpyridine (**23**; Aldrich; 50.0 g, 0.295 mol), sodamide (Aldrich; 19.0 g, 0.487 mol), and *p*-cymene[22] (350 mL) were heated with stirring at 155–160 °C. After 1 h (the mixture had become dark brown and difficult to stir), a further 50 mL of *p*-cymene was added; heating (with stirring) was continued for 8 h. The mixture was allowed to cool, and water (100 mL) was added slowly followed by concentrated hydrochloric acid (50 mL). The layers were separated and the organic layer was extracted with 50 mL of 10% HCl. The aqueous layers were combined, washed once with ether (100 mL), and then made strongly basic with solid potassium hydroxide, during which time a black oil separated which was extracted into CH_2Cl_2; the extract was dried (Na_2SO_4) and evaporated to give a black solid. Kugelrohr distillation (0.1 Torr, 145–160 °C) gave a mixture of aminobenzylpyridine isomers as a yellow crystalline solid (37 g). The solid was recrystallized from ether giving colorless needles, which were filtered off and identified by ^1H NMR as unwanted 2-amino-3-benzylpyridine: 17.0 g; mp 123–124 °C; ^1H NMR ($CDCl_3$) δ 3.83 (2 H, s), 4.35 (2 H, br s), 6.65–6.70 (1 H, dd, J = 7.3, 5.0 Hz), 7.16–7.19 (2 H, m), 7.25–7.34 (4 H, m), 8.00–8.03 (1 H, dd, J = 5.0, 0.9 Hz). Anal. Calcd for $C_{12}H_{12}N_2$: C, 78.22; H, 6.57; N, 15.21. Found: C, 78.40; H, 6.52; N, 15.21.

The mother liquor from above was concentrated and purified by flash column chromatography on silica gel, eluting with 49:1 CH_2Cl_2/MeOH, giving additional 2-amino-3-benzylpyridine (2.8 g, total yield 19.8 g, 36%) and 2-amino-5-benzylpyridine (**24**; 9.4 g, 17%) as colorless shiny platelets: mp 75–76 °C (Et_2O/petroleum ether); ^1H NMR ($CDCl_3$) δ 3.83 (2 H, s), 4.38 (2 H, br s), 6.42–6.45 (1 H, d, J = 8.5 Hz), 7.15–7.31

(32) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(6 H, m), 7.96 (1 H, d, J = 2.4 Hz); IR (KBr) ν 3415 (br), 3310 (br), 3160 (br), 3027, 2905, 1641, 1568 cm^{-1}; mass spectrum, m/e (relative intensity) 185 (14), 184 (100, M^+), 183 (86). Anal. Calcd for $C_{12}H_{12}N_2$: C, 78.22; H, 6.57; N, 15.21. Found: C, 78.10; H, 6.48; N, 15.09.

2-Amino-5-benzyl-3-bromopyridine (25). To a stirred solution of **24** (3.00 g, 16.3 mmol) in CH_2Cl_2 (50 mL) cooled to 0 °C under argon was added bromine (2.6 g, 1.0 equiv) dropwise. The bromine decolorized immediately and the mixture was left stirring for 30 min, during which time a yellow solid precipitated. The suspension was shaken with 5% aqueous sodium bicarbonate (100 mL); the organic layer was then dried (Na_2SO_4) and evaporated giving **25**: 4.27 g, 100%; mp 98–100.5 °C; ^1H NMR ($CDCl_3$) δ 3.82 (2 H, s), 4.82 (2 H, br s), 7.15–7.33 (5 H, m), 7.46 (1 H, d, J = 2.1 Hz), 7.90 (1 H, d, J = 2.1 Hz); IR (KBr) ν 3475, 3282, 3154 (br), 1628, 1484 cm^{-1}; mass spectrum, m/e (relative intensity) 264 (98, M^+), 263 (83), 262 (100, M^+).

2-(Acetylamino)-7-methyl-1,8-naphthyridine (26). 2-Amino-7-methyl-1,8-naphthyridine[33] (1.00 g, 6.28 mmol) was suspended in 10 mL of acetic anhydride and stirred at 140 °C for 1 h. After cooling, the solid was filtered off and washed with ether to give 0.51 g of pure **26**. The filtrate and wash were combined and concentrated; the residue was purified by flash column chromatography on silica gel, eluting with 9:1 CH_2Cl_2/MeOH to give an additional 0.29 g (total yield 63%) of **26**: mp 278–280 °C (lit.[33] mp 279–281 °C); ^1H NMR ($CDCl_3$) δ 2.23 (3 H, s), 2.76 (3 H, s), 7.28 (1 H, d, J = 8.4 Hz), 8.01 (1 H, d, J = 8.4 Hz), 8.14 (1 H, d, J = 8.7 Hz), 8.46 (1 H, d, J = 8.7 Hz), 8.73 (1 H, s, br).

6-Amino-3-benzyl-5-(3-biphenylyl)pyrid-2-one (27). 2-(Acetylamino)-5-benzyl-3-(3-biphenylyl)pyridine N-oxide (**32**; 150 mg, 0.38 mmol) was added in one portion to acetic anhydride (3 mL) being rapidly stirred under argon at 140 °C. The mixture became dark brown almost immediately and stirring was continued at 140 °C under argon for a further 3.5 h. After cooling, the acetic anhydride was removed in vacuo on a Kugelrohr and the residue was purified by preparative TLC (1000-μm plate) by developing with 49:1 CH_2Cl_2/MeOH to give an orange-brown oil (87 mg). Without further purification, MeOH (3 mL) and sodium carbonate (262 mg, 1.52 mmol) were added and the solution was stirred overnight at room temperature. The MeOH was evaporated in vacuo and the residue was taken up in CH_2Cl_2, washed with 5% aqueous sodium bicarbonate, dried (Na_2SO_4), and evaporated to give a brown oil, which was purified by preparative TLC (1000-μm plate) developing with 9:1 CH_2Cl_2/MeOH to give **27** (25 mg, 19%) as a light yellow oil, which foamed under vacuum to a crystalline solid: mp 218–219 °C dec; ^1H NMR ($CDCl_3$) δ 3.77 (2 H, s), 5.30 (2 H, br s), 7.01–7.07 (1 H, m), 7.16–7.60 (14 H, m), 11.94 (1 H, br s); IR (KBr) ν 3490, 3390, 2958, 2925, 2854, 2692, (br) 1631, 1604 cm^{-1}.

2-(Acetylamino)-5-benzyl-3-bromopyridine N-Oxide (30). To a solution of 2-(acetylamino)-5-benzyl-3-bromopyridine (**19**; 1.00 g, 3.26 mmol) in CH_2Cl_2 (20 mL) was added m-chloroperoxybenzoic acid (Aldrich, 80%; 1.2 g, 6.9 mmol), and the mixture was stirred under argon at room temperature overnight. The yellow solution was diluted with CH_2Cl_2 (30 mL) and washed with 5% aqueous sodium bicarbonate (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase and extracts were combined, dried (Na_2SO_4), and evaporated to give a yellow oil, which was dissolved in a minimum volume of CH_2Cl_2 and triturated with ether to give **30** (850 mg, 81%) as a white crystalline solid: mp 158–159 °C dec; ^1H NMR ($CDCl_3$) δ 2.27 (3 H, s), 3.89 (2 H, s), 7.15–7.18 (2 H, m), 7.27–7.35 (3 H, m), 7.37 (1 H, d, J = 1.7 Hz), 8.04 (1 H, d, J = 1.7 Hz), 8.56 (1 H, br s); IR (KBr) ν 3135 (br), 2938 (br), 1714 cm^{-1}; mass spectrum, m/e (relative intensity) 322 (5, M^+), 320 (6, M^+), 280 (95), 278 (100). Anal. Calcd for $C_{14}H_{13}N_2O_2Br$: C, 52.35; H, 4.08; N, 8.72. Found: C, 52.39; H, 4.02; N, 8.53.

3-Biphenylboronic acid (31). To a solution of 3-bromobiphenyl (3.00 g, 12.9 mmol, Aldrich) in anhydrous THF (50 mL) at −78 °C under argon was added n-butyllithium (5.40 mL of a 2.5 M solution in hexanes, 13.5 mmol; Aldrich). The mixture was stirred for 45 min at −78 °C, during which time the solution became light yellow. Trimethyl borate (1.53 mL, 13.5 mmol) was added dropwise and the solution was stirred for 1 h at −78 °C followed by 3 h at room temperature and then quenched with 10% hydrochloric acid (25 mL).[17] The mixture was diluted with ethyl acetate (50 mL), and the organic layer was separated. The aqueous layer was exhaustively extracted with further portions of ethyl acetate; the organic phases were combined, dried (Na_2SO_4), and evaporated to give a white solid, which was suspended in petroleum ether and filtered giving **31**: 1.60 g, 63%; mp 192–195 °C; ^1H NMR ($CDCl_3$) δ 7.39–7.64 (4 H, m), 7.70–7.73 (2 H, d, J = 7.3 Hz), 7.83–7.86 (1 H, d, J = 7.8 Hz), 8.25–8.27 (1 H, d, J = 7.8 Hz), 8.48 (1 H, s); IR (KBr) ν 3261 (br), 3057, 1602, 1412, 1349, 758, 723, 702 cm^{-1}; mass spectrum, m/e (relative intensity), 198 (1, M^+), 180 (100). This material was used without further purification.

2-(Acetylamino)-5-benzyl-3-(3-biphenylyl)pyridine N-Oxide (32). 2-(Acetylamino)-5-benzyl-3-bromopyridine N-oxide (**30**; 400 mg, 1.25 mmol), 3-biphenylboronic acid (**31**; 271 mg, 1.37 mmol), and sodium carbonate (264 mg, 2.74 mmol) were dissolved in a mixture of toluene (18 mL), water (3 mL), and ethanol (6 mL). With stirring was added tetrakis(triphenylphosphine)palladium(0) (72 mg, 5 mol%), and the mixture was heated at 90–95 °C under argon for 17 h, during which time the organic layer became dark brown. On being cooled, the mixture was diluted with CH_2Cl_2 and the organic layer was separated, washed with 5% aqueous sodium bicarbonate, dried (Na_2SO_4), and evaporated to give a yellow oil. Purification by flash column chromatography on silica gel, eluting with 95:5 CH_2Cl_2/MeOH, gave 2-(acetylamino)-5-benzyl-3-(3-biphenylyl)pyridine N-oxide (**32**; 470 mg, 1.19 mmol, 95%) as a white, foamy solid: mp 93–95 °C; ^1H NMR ($CDCl_3$) δ 2.05 (3 H, s), 3.96 (2 H, s), 7.19–7.61 (14 H, m), 8.1 (1 H, d, J = 1.8 Hz), 8.90 (1 H, br s); IR (KBr) ν 3226 (br), 3149 (br), 3064, 1701, 1494 cm^{-1}; mass spectrum, m/e (relative intensity), 394 (1, M^+), 378 (5), 91 (100).

2-(Acetylamino)-7-(chloromethyl)-1,8-naphthyridine (33). 2-(Acetylamino)-7-methyl-1,8-naphthyridine (**26**; 200 mg, 0.99 mmol) and Na_2CO_3 (210 mg, 1.98 mmol) in 20 mL of CCl_4 were stirred at 60 °C. A slow stream of chlorine gas was passed through for 5.5 h at that temperature; the reaction mixture was then stirred overnight at room temperature. Aqueous Na_2CO_3 was added, the layers were separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined organic phases were dried (Na_2SO_4) and evaporated. The residue was separated by preparative TLC (1000-μm plate, 19:1 CH_2Cl_2/MeOH) to give 30 mg of crude **33**, which was further purified by preparative TLC (1000-μm plate, EtOAc) to give **33** (15 mg, 6.8%); recrystallization from CH_2Cl_2/MeOH gave a white solid, which decomposed at 189–191 °C, without melting, to give a gray-green solid: mp >300 °C; ^1H NMR ($CDCl_3$) δ 2.29 (3 H, s), 4.85 (2 H, s), 7.65 (1 H, d, J = 8.3 Hz), 8.21 (1 H, d, J = 8.3 Hz), 8.22 (1 H, d, J = 8.8 Hz), 8.46 (1 H, br s), 8.54 (1 H, d, J = 8.8 Hz); mass spectrum, m/e (relative intensity), 237 (8, M^+), 235 (25, M^+), 193 (100); exact mass calcd for $C_{11}H_{11}N_3OCl$ $[M + H]^+$ 236.0590, found 236.0597.

3-[5-(6-Amino-3-benzyl-2-oxopyridyl)]-5-[3-(2-amino-5-oxo-7-phenyl-1,8-naphthyridinyl)]biphenyl (41). To a solution of crude (containing some deacetylated material; see procedure for its preparation) **49** (96 mg, 0.14 mmol) in acetic acid (10 mL) was added an aqueous solution of hydrogen bromide (Fisher, 48%; 10 mL), and the mixture was heated at reflux for 1.5 h. After cooling, the solvents were evaporated and the residue was treated with 5% aqueous sodium bicarbonate and extracted with CH_2Cl_2; the extracts were dried (Na_2SO_4) and evaporated to give a yellow solid. Purification by flash column chromatography on silica gel, eluting with 9:1 CH_2Cl_2/MeOH, gave **41** (60 mg, 75%) as a pale yellow powder, mp 268–270 °C (dec) after recrystallization from CH_2Cl_2/CH_3OH/Et_2O: ^1H NMR (DMSO-d_6) δ 3.62 (2 H, s), 5.82 (2 H, br s), 6.18 (1 H, s), 6.59 (2 H, s, br), 7.09–7.53 (16 H, m), 7.75–7.79 (3 H, m), 7.98 (1 H, s), 10.72 (1 H, br s), 11.66 (1 H, br s); IR (KBr) ν 3451, 3395 (br), 3303, 3191, 3057, 2921, 1618 cm^{-1}; exact mass calcd for $C_{38}H_{30}N_5O_2$ $[M + H]^+$ 588.2399, found 588.2412.

7-(Bromomethyl)-1,8-naphthyridin-2-one (42). 7-Methyl-1,8-naphthyridin-2-one (**59**;[33] 2.00 g, 12.5 mmol), N-bromosuccinimide (Aldrich; 4.45 g, 25.0 mmol), and benzoyl peroxide (Aldrich, 97%; 90 mg) in anhydrous chloroform (Aldrich, stabilized with 0.5–1.0% ethanol; 150 mL) were refluxed with stirring for 6.5 h while being irradiated with a 250-W Westinghouse household Heat Ray infrared lamp. After being cooled, the reaction mixture was washed with water (4 × 100 mL), dried (Na_2SO_4), and evaporated to give a solid. Purification by flash column chromatography on silica gel (EtOAc) gave **42** (242 mg, 8%) as a white crystalline solid, mp 199–200 °C after recrystallization from CH_2Cl_2/Et_2O: ^1H NMR ($CDCl_3$) δ 4.63 (2 H, s), 6.73 (1 H, d, J = 9.6 Hz), 7.33 (1 H, d, J = 7.8 Hz), 7.71 (1 H, d, J = 9.6 Hz), 7.90 (1 H, d, J = 7.8 Hz), 10.11 (1 H, br s); IR (KBr) ν 3472 (br), 3135, 3057, 2994, 2945, 2861, 1653, 1601 cm^{-1}; mass spectrum, m/e (relative intensity), 241 (24), 240 (94, M^+), 239 (27), 238 (94, M^+), 159 (100). Anal. Calcd for $C_9H_7N_2OBr$: C, 45.22; H, 2.95; N, 11.72. Found: C, 45.53; H, 2.81; N, 11.55.

2-(Acetylamino)-5-benzyl-3-bromo-6-methoxypyridine (46). 6-(Acetylamino)-3-benzyl-5-bromopyrid-2-one (**52**; 2.00 g, 6.25 mmol) and silver(I) carbonate (860 mg, 3.13 mmol) were suspended in benzene (5 mL) and iodomethane (1.95 mL, 5.0 equiv) was added. The mixture was stirred rapidly at ambient temperature under argon in the dark for 4 days. The silver salts were filtered off and washed with CH_2Cl_2, and the combined filtrate and washings were evaporated to give an oily residue, which was purified by flash column chromatography on silica gel eluting with 1:1 ethyl acetate/petroleum ether, giving **46** (1.35 g, 65%) as a white solid: mp 140–141 °C; ^1H NMR ($CDCl_3$) δ 2.52 (3 H, s), 3.84 (2 H,

(33) Brown, E. V. *J. Org. Chem.* **1965**, *30*, 1607–1610.

s), 3.92 (3 H, s), 7.17–7.35 (5 H, m), 7.39 (1 H, s), 7.72 (1 H, br s); IR (KBr) ν 3240, 1278, 1243 cm^{-1}; mass spectrum, *m/e* (relative intensity), 336 (82, M^+), 334 (82, M^+), 255 (100). Anal. Calcd for $C_{15}H_{15}N_2O_2Br$: C, 53.74; H, 4.51; N, 8.36. Found: C, 53.82; H, 4.41; N, 8.15.

3-(Trimethylstannyl)-5-[3-[2-(acetylamino)-5-benzyl-6-methoxypyridyl]]biphenyl (47). A mixture of **18** (1.43 g, 3.00 mmol), **46** (500 mg, 1.5 mmol), and bis(triphenylphosphine)palladium(II) chloride (21 mg, 0.030 mmol) in toluene was heated at 95–100 °C for 2 h under argon with stirring. The solvent was removed in vacuo and the residue was purified directly by flash column chromatography on silica gel, eluting with 39:1 CH_2Cl_2/MeOH to give **47** (355 mg, 41%) as a white foamy solid: mp 133–135 °C; ^1H NMR ($CDCl_3$) δ 0.33 (9 H, s), 2.53 (3 H, s), 3.92 (2 H, s), 3.99 (3 H, s), 7.25–7.66 (15 H, m); IR (KBr) ν 3458 (br), 3395, 3268 (br), 3029, 2980, 1672, 1609, 1581 cm^{-1}; mass spectrum, *m/e* (relative intensity), 575 (3), 573 (3), 572 (5), 571 (18, $[M - H]^+$ for principal Sn isotope), 570 (9), 569 (13), 568 (6), 567 (8), 390 (100).

2-(Acetylamino)-3-bromo-5-methoxy-7-phenyl-1,8-naphthyridine (48). 2-Amino-3-bromo-5-methoxy-7-phenyl-1,8-naphthyridine (**58**; 400 mg, 1.21 mmol) and acetic anhydride (30 mL) were heated with stirring under argon at 70–80 °C for 1.5 h. The acetic anhydride was removed in vacuo on a Kugelrohr and the residue was purified by flash column chromatography on silica gel, eluting with 95:5 CH_2Cl_2/MeOH to give **48** (410 mg, 91%) as a yellow powdery solid: mp 209–210 °C; ^1H NMR ($CDCl_3$) δ 2.79 (3 H, s), 4.14 (3 H, s), 7.22 (1 H, s), 7.50–7.53 (3 H, m), 8.15–8.18 (2 H, dd, J = 8.1, 2.1 Hz), 8.22 (1 H, br s), 8.69 (1 H, s); IR (KBr) ν 3374, 2938, 1686, 1595 cm^{-1}; mass spectrum, *m/e* (relative intensity), 373 (11, M^+), 371 (11, M^+), 292 (100). Anal. Calcd for $C_{17}H_{14}N_3O_2Br$: C, 54.85; H, 3.79; N, 11.29. Found: C, 55.10; H, 3.78; N, 11.24.

3-[3-[2-(Acetylamino)-5-benzyl-6-methoxypyridyl]]-5-[3-[2-(acetylamino)-5-methoxy-7-phenyl-1,8-naphthyridyl]]biphenyl (49). A mixture of **47** (221 mg, 0.39 mmol), **48** (120 mg, 0.32 mmol), and bis(triphenylphosphine)palladium(II) chloride (11 mg, 0.020 mmol) in toluene (4 mL) was heated at 105–110 °C for 16 h under argon with stirring in a sealed tube. The solvent was evaporated and the residue was purified by flash column chromatography on silica gel (29:1 CH_2Cl_2/CH_3OH) to give a yellow solid (96 mg), which consisted of a mixture of the required product plus deacetylated product (confirmed by ^1H NMR). This mixture can be used in the next step (the preparation of **41**) without further purification.

A small portion of the mixture (15 mg) and acetic anhydride (1 mL) were stirred at 70 °C for 1 h. The acetic anhydride was removed in vacuo on a Kugelrohr and the residue was recrystallized from CH_2Cl_2/ether to give **49** as a pale yellow solid: mp 156–158 °C; ^1H NMR ($CDCl_3$) δ 2.45 (3 H, s), 2.60 (3 H, s), 3.94 (2 H, s), 4.00 (3 H, s), 4.13 (3 H, s), 7.20–7.66 (19 H, m), 7.92 (1 H, s), 8.21 (1 H, s), 8.24 (1 H, s), 8.46 (1 H, s); IR (KBr) ν 3465 (br), 3388, 3071, 3029, 2945, 1679, 1602 cm^{-1}; exact mass calcd for $C_{44}H_{38}N_5O_4$ $[M + H]^+$ 700.2923, found 700.2902.

2-Amino-5-benzyl-3-bromopyridine *N*-Oxide (50). Pyridine **25** (5.00 g, 19.0 mmol) and *m*-chloropeoxybenzoic acid (Aldrich, 80%; 4.10 g, 23.7 mmol) in CH_2Cl_2 (100 mL) were stirred at room temperature overnight under argon, giving an orange solution. Solid potassium carbonate (5.0 g) was added, and the suspension was heated on a steam bath for 10 min and then filtered; the filtrate was evaporated to give **50** as an orange solid (4.77 g, 90%), which was ordinarily used without further purification. An analytical sample was obtained as a white solid, mp 97–98 °C, by recrystallization from ethyl acetate/petroleum ether: ^1H NMR ($CDCl_3$) δ 3.81 (2 H, s), 5.84 (2 H, br s), 7.14–7.20 (3 H, m), 7.26–7.35 (3 H, m), 7.94 (1 H, s); IR (KBr) ν 3367, 3219, 3163, 3050 (br), 1625 cm^{-1}; mass spectrum *m/e* (relative intensity), 280 (97, M^+), 278 (100, M^+). Anal. Calcd for $C_{12}H_{11}N_2OBr$: C, 51.63; H, 3.97; N, 10.04. Found: C, 51.73; H, 3.85; N, 9.92.

6-Acetoxy-2-(*N*,*N*-diacetylamino)-5-benzyl-3-bromopyridine (51). Crude **50** (4.77 g, 17.1 mmol) and acetic anhydride (100 mL) were heated under an atmosphere of argon at 140 °C for 4 h with stirring, during which time the mixture became dark brown. The acetic anhydride was removed in vacuo on a Kugelrohr and the resulting brown oil was purified by flash column chromatography on silica gel, eluting with 1:1 ethyl acetate/petroleum ether, to give **51** (5.05 g, 73%) as an orange oil: ^1H NMR ($CDCl_3$) δ 2.28 (3 H, s), 2.29 (6 H, s), 3.92 (2 H, s), 7.17–7.19 (2 H, m), 7.30–7.40 (3 H, m), 7.74 (1 H, s).

6-(Acetylamino)-3-benzyl-5-bromopyrid-2-one (52). Acetoxypyridine **51** (5.05 g, 12.4 mmol) and sodium carbonate (5.00 g, 47.0 mmol) in MeOH (100 mL) were stirred rapidly for 30 min at room temperature. The MeOH was evaporated and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The layers were separated and the aqueous layer was extracted exhaustively with ethyl acetate; the combined organic phases were dried (Na_2SO_4) and evaporated to give a white solid, which was suspended in ether and filtered giving **52**: 2.10 g, 50%; mp 182–183 °C dec; ^1H NMR ($CDCl_3$) δ 2.28 (3 H, s), 3.81 (2 H, s), 7.03 (1 H, s), 7.21–7.34 (5 H, m), 7.68 (1 H, br s); IR (KBr) ν 3205 (br), 3170 (br), 1644, 1588 cm^{-1}; mass spectrum, *m/e* (relative intensity), 322 (57, M^+), 320 (59, M^+), 280 (95), 278 (100). Anal. Calcd for $C_{14}H_{13}N_2O_2Br$: C, 52.35; H, 4.08; N, 8.72. Found: C, 52.33; H, 3.95; N, 8.56.

2-Amino-7-phenyl-1,8-naphthyridin-5-one (53).[34] 2,6-Diaminopyridine (Aldrich, 2.18 g, 0.020 mol), ethyl benzoylacetate (Aldrich, 90%, 5.76 g, 0.030 mol), and diphenyl ether (50 mL) were heated at 130 °C with stirring for 40 min and then heated at reflux for 2 h. After cooling, the yellow-brown precipitate was filtered and washed with petroleum ether to give 3.42 g of crude product, which was purified by flash column chromatography on silica gel eluting with CH_2Cl_2/MeOH (9:1) to give **53** (2.96 g, 62.4%) as a foamy brown solid, mp 85–120 °C (but essentially pure by ^1H NMR, and used without further purification): ^1H NMR (DMSO-d_6) δ 6.15 (1 H, s), 6.50 (1 H, d, J = 8.6 Hz), 6.77 (2 H, br s), 7.49–7.52 (3 H, m), 7.43–7.77 (2 H, m), 8.01 (1 H, d, J = 8.6 Hz), 11.51 (1 H, br s); IR (KBr) ν 3318 (br), 3177 (br), 1616, 1532 cm^{-1}; mass spectrum, *m/e* (relative intensity), 238 (14), 237 (84, M^+), 209 (100).

2-(Isobutyroylamino)-7-phenyl-1,8-naphthyridin-5-one (55). A solution of **53** (7.40 g, 30.6 mmol) in isobutyric anhydride (100 mL) was heated at 50–60 °C for 3 h under argon. The excess anhydride was removed in vacuo on a Kugelrohr to give a brown oil (crude **54**), which was not purified [^1H NMR ($CDCl_3$) δ 1.31 (6 H, d, J = 7 Hz), 2.67 (1 H, septet, J = 7 Hz), 6.54 (1 H, s), 7.48–7.56 (3 H, m), 7.83 (1 H, s), 8.23–8.27 (2 H, m), 8.28 (1 H, d, J = 9 Hz), 8.57 (1 H, d, J = 9 Hz), 8.97 (1 H, br s)]. Methanol (100 mL) was then added to the flask followed by sodium carbonate (7.50 g, 70.1 mmol) and the mixture was stirred rapidly for approximately 1.5 h, or until a thick yellow suspension formed. The MeOH was evaporated to dryness and the residue was extracted with $CHCl_3$; the extracts were washed with water, dried (Na_2SO_4), and evaporated to give a yellow solid. Redissolving the solid in the minimum volume of CH_2Cl_2 and triturating with ether gave **55** (6.50 g, 70%) as a creamy white solid: mp 266–267 °C; ^1H NMR ($CDCl_3$, warm to dissolve) δ 1.27–1.29 (6 H, d, J = 6.9 Hz), 2.55–2.64 (1 H, septet, J = 6.9 Hz), 6.52 (1 H, s), 7.50–7.53 (3 H, m), 7.62–7.65 (2 H, m), 8.10 (1 H, br s), 8.22–8.25 (1 H, d, J = 8.8 Hz), 8.60–8.63 (1 H, d, J = 8.8 Hz), 8.94 (1 H, br s); IR (KBr) ν 3416 (br), 3233–3064 (br), 2973, 1707, 1616 cm^{-1}; mass spectrum, *m/e* (relative intensity), 307 (51, M^+), 237 (100).

2-(Isobutyroylamino)-5-methoxy-7-phenyl-1,8-naphthyridine (56). To a suspension of **55** (2.00 g, 6.50 mmol) and silver(I) oxide [0.75 g, 3.25 mmol (0.50 equiv)] in acetone (50 mL) was added iodomethane (2.0 mL, 5.0 equiv), and the mixture was heated at reflux under argon with stirring. Initially, the starting material was insoluble, but gradually over 2 h the creamy colored solid disappeared to give a bright yellow suspension of silver iodide. The precipitate was filtered off and washed with CH_2Cl_2, and the combined filtrate and washings were evaporated. The residual oil was purified by flash column chromatography on silica gel, eluting with 19:1 CH_2Cl_2/MeOH to give **56** (757 mg, 36%) as a shiny solid foam: mp 194–195 °C; ^1H NMR ($CDCl_3$) δ 1.29 (6 H, d, J = 4.0 Hz), 2.62 (1 H, septet, J = 4.0 Hz), 4.13 (3 H, s), 7.18 (1 H, s), 7.47–7.49 (3 H, m), 8.20 (2 H, dd, J = 7.8, 1.9 Hz), 8.44 (1 H, d, J = 9.0 Hz), 8.48 (1 H, br s), 8.51 (1 H, d, J = 9.0 Hz); IR (KBr) ν 3177–3008 (br), 2973, 1699, 1602 cm^{-1}; mass spectrum, *m/e* (relative intensity), 322 (16), 321 (73, M^+), 251 (100). Anal. Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08. Found: C, 70.73; H, 5.93; N, 12.95.

2-Amino-5-methoxy-7-phenyl-1,8-naphthyridine (57). To a solution of **56** (1.56 g, 4.86 mmol) in MeOH (30 mL) was added 10% aqueous sodium hydroxide (10 mL), and the mixture was stirred overnight at room temperature. The mixture was evaporated to dryness and CH_2Cl_2 was added, and the solution was washed with water, dried (Na_2SO_4), and evaporated to a small volume. Trituration of the CH_2Cl_2 solution with petroleum ether gave a yellow crystalline solid, which was filtered off and dried giving **57**: 1.10 g, 91%; mp 247–248 °C; ^1H NMR ($CDCl_3$) δ 4.08 (3 H, s), 5.03 (2 H, br s), 6.69 (1 H, d, J = 8.8 Hz), 7.09 (1 H, s), 7.45–7.50 (3 H, m), 8.20–8.24 (2 H, dd, J = 7.3, 1.5 Hz), 8.23 (1 H, d, J = 8.8 Hz); IR (KBr) ν 3332 (br), 3128 (br), 1613, 1593 cm^{-1}; mass spectrum *m/e* (relative intensity), 252 (16), 251 (100, M^+), 250 (89). Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.34; H, 5.11; N, 16.55.

(34) The procedure is based on one described in: Barlin, G. B.; Tan, W. L. *Aust. J. Chem.* **1984**, *37*, 1065–1073.

(35) Cf.: Skoog, D. A.; West, D. M.; Holler, F. J. *Fundamentals of Analytical Chemistry*, 5th ed.; Saunders: Philadelphia, 1988; pp 526–527. Bovey, F. A. *Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; Academic Press: New York, 1988; p 296. For a comprehensive treatment of the determination of binding constant, see: Connors, K. A. *Binding Constants*; Wiley-Interscience: New York, 1987.

2-Amino-3-bromo-5-methoxy-7-phenyl-1,8-naphthyridine (58). To a stirred solution of **57** (500 mg, 2.00 mmol) and iodine[30] (50 mg) in anhydrous dichloromethane was added bromine (150 μL, 2.0 mmol) dropwise at ambient temperature. The first few drops of bromine were decolorized immediately; on continued stirring, a solid precipitated. After 24 h, the mixture was shaken with 5% aqueous sodium bicarbonate and the organic phase was separated, dried (Na_2SO_4), and evaporated; purification by flash column chromatography on silica gel, eluting with 19:1 CH_2Cl_2/MeOH, gave **58** (400 mg, 61%) as a yellow solid: mp 241–242 °C; ^1H NMR ($CDCl_3$) δ 4.09 (3 H, s), 5.61 (2 H, br s), 7.10 (1 H, s), 7.46–7.51 (3 H, m), 8.18–8.21 (2 H, dd, J = 7.8, 1.5 Hz), 8.49 (1 H, s); IR (KBr) ν 3261 (br), 3114 (br), 1615, 1595 cm^{-1}; mass spectrum, m/e (relative intensity), 331 (98, M^+), 330 (100), 329 (99, M^+), 328 (97).

2-Amino-3-(biphenyl-3-yl)-7-phenyl-1,8-naphthyridine-5-one (60). In a thick-walled glass tube containing a stir bar were placed **48** (30 mg, 0.081 mmol), **31** (18 mg, 0.090 mmol), and sodium carbonate (18 mg, 0.17 mmol) in a mixture of toluene (1 mL), ethanol (250 μL), and water (250 μL). To this two-phase system was added tetrakis(triphenylphosphine)palladium(0) (5 mg, 5 mol%) and the tube was sealed and heated in an oil bath at 90–95 °C for 18 h with stirring, during which time the organic layer became dark brown. After being cooled, the contents were diluted with CH_2Cl_2, washed with 5% aqueous sodium bicarbonate, dried (Na_2SO_4), and evaporated to give an oily solid, which was heated with acetic anhydride (2 mL) for 1 h at 75 °C. The excess acetic anhydride was evaporated in vacuo and the product was purified by preparative TLC (1000-μm plate) by developing with 19:1 CH_2Cl_2/MeOH to give 2-(acetylamino)-3-(biphenyl-3-yl)-5-methoxy-7-phenyl-1,8-naphthyridine (33 mg, 91%) as a white crystalline solid: ^1H NMR ($CDCl_3$) δ 2.67 (3 H, s), 4.07 (3 H, s), 7.24 (1 H, s), 7.36–7.72 (12 H, m), 8.08 (1 H, br s), 8.21 (2 H, dd, J = 8.2, 1.8 Hz), 8.43 (1 H, s). This was used without further purification.

To a stirred solution of 2-(acetylamino)-3-(biphenyl-3-yl)-5-methoxy-7-phenyl-1,8-naphthyridine (68 mg, 0.15 mmol) in acetic acid (2 mL) was added an aqueous solution of hydrogen bromide (Fisher, 48%; 2 mL) and the mixture was heated at reflux for 3 h. On being cooled, the solution was diluted with water (5 mL), neutralized to pH 7 with 1 M sodium hydroxide solution, and extracted with CH_2Cl_2; the CH_2Cl_2 extracts were dried (Na_2SO_4) and evaporated to give a white solid, which was suspended in ether, filtered, and dried, giving **60** (39 mg, 68%) as a powdery white solid: mp 295–296 °C dec; ^1H NMR ($CDCl_3$, warm to dissolve) δ 5.13 (2 H, br s), 6.53 (1 H, s), 7.39–7.41 (1 H, d, J = 8.4 Hz), 7.45–7.72 (13 H, m), 8.38 (1 H, s), 8.54 (1 H, br s); IR (KBr) ν 3332 (br), 3205 (br), 3064, 1619 cm^{-1}; mass spectrum, m/e (relative intensity), 390 (16), 389 (68, M^+), 154 (100).

Measurement of Binding Constants. Two ^1H NMR methods were employed for obtaining the data needed for calculation of binding constants. All measurements of chemical shifts were obtained in deuteriochloroform at an NMR probe temperature of 25 °C (±1 °C) measured downfield from internal tetramethylsilane. In all cases exchange was observed to be rapid on the NMR time scale;[26] i.e., only average spectra were observed, not superpositions of spectra of bound and unbound species.

General Method A. Samples corresponding to a 1.0:1.0 molar ratio of the binding partners were accurately weighed into an NMR tube and 0.500 (±0.005) mL of $CDCl_3$ (0.03% TMS)[36] was added (since complexes are often more soluble than individual components, slowly soluble compounds often required prolonged shaking to give a completely homogeneous solution) to give a stock solution of 0.080 M concentration in each component (since <5 mg of each partner was used, the assumption of a final volume of 0.500 mL is accurate to within 2%). The tube was placed in the NMR probe and the spectrum was recorded, measuring the chemical shifts of selected protons (normally those were the AcN*H* protons, since their chemical shifts were most sensitive to the degree of binding). Progressively more dilute solutions were made, using aliquots of the original stock solution and diluting them with appropriate amounts of $CDCl_3$ (0.03% TMS). Typically, spectra of 12–14 different solutions with concentrations ranging from 0.080 to 0.000 250 M (lower limit of NMR sensitivity) were recorded. At the concentrations studied, self-association of individual components was generally negligible [parallel dilutions of $CDCl_3$ solutions of individual partners led to relatively insignificant (<0.4 ppm) Δδ's compared to δΔ's of 2–4 ppm associated with complex formation]. A plot of concentration versus chemical shift of the selected protons (two protons were monitored independently to ensure internal consistency) allows extrapolation to give the chemical shift of the proton at 100% binding and the percentage of template–substrate complex in $CDCl_3$ at any concentration. Binding constants were calculated by the mole fraction method.[35]

Table I

[10] = [27]/M	δ H_A	δ H_B	δ H_A in the absence of **27**
0.040	11.828	6.430	8.558
0.020	11.615	6.380	8.439
0.010	11.431	6.298	8.361
0.0050	11.239	6.208	8.320
0.0040	11.145	6.158	
0.0020	10.923	6.050	8.297
0.00125	10.760	5.972	
0.0010	10.671	5.911	8.291
0.000625	10.443	5.808	
0.00050	10.359	5.755	8.290
0.000375	10.130	5.625	
0.00025	10.065	5.584	
0.0000	8.29[a]		

[a] Extrapolated value.

A typical set of data is provided in Table I. The data in Table I are for the binding of **10** with **27**; H_A is the AcN*H* proton of **10**, H_B is the NH_2 protons of **27**; [**10**] and [**27**] are the total (bound and unbound) molar concentrations of each.

General Method B. The ^1H NMR spectrum of the host at known concentration in $CDCl_3$ (usually 0.080 M) was recorded to obtain the unbound chemical shifts of selected protons (see method A). To the NMR tube was added approximately 1 equiv (exact amount checked by ^1H NMR integration to ensure accuracy of transfer) of the substrate partner and the ^1H NMR spectrum was recorded, measuring the change in the δ's of the selected protons. The maximum chemical shifts of selected protons of the template when 100% bound to the substrate were obtained by adding additional equivalents of substrate partner in increments (normally up to 5–7 equiv) to the tube, and the spectrum was recorded each time until the chemical shifts of the template protons no longer changed upon further addition of substrate. Binding constants were calculated as in method A.

Binding constants for selected complexes were checked by both general methods; the two methods gave consistent results.

Kinetics. Initial rates of reaction were measured by ^1H NMR in $CDCl_3$ at an NMR probe temperature of 25 °C (±1 °C) with the concentration of template and substrates both initially at 0.0040 M.[23] *sym*-Tetrachloroethane (which has a chemical shift of 5.96 ppm, conveniently adjacent to, but clear of, protons corresponding to either the substrates or templates) was chosen as an internal standard. The *sym*-tetrachloroethane (1–2 μL) was added directly to the NMR tube and the reaction was monitored by integration of the bromomethylene protons of the respective substrates (**11** and **42**) versus the standard over a period of hours. As an internal control, the integration of the methylene protons of the amine **10** was also monitored, but the peak is broader and, therefore, the integration is less accurate. Since products **14** and **45** precipitated (as their unbound hydrobromide salts), it was not possible to measure product formation by NMR. Integration of the benzyl CH_2 peak of template **9** (or **41**, as relevant) indicated no change in its concentration during the course of the reaction. The preacquisition delay (PAD) utility available on the software of the Varian XL-300 NMR spectrometer was used to record spectra of the reaction at 5-min intervals for the first hour, then at 20-min intervals for the next 2 h, and then at 1-h intervals thereafter. At the concentrations employed (0.0040 M), an acceptable signal/noise ratio for relatively accurate integration of the spectra could be obtained with 64 transients (more transients would have given more accurate integrations, but at the expense of less accuracy in the measurement of reaction time). Plots of reaction progress (consumption of bromomethylene substrates) with time were obtained using Cricket Graph[37] for the Macintosh. Rate enhancements were calculated by taking a ratio of the initial rates of reactions in the presence of the template versus the control reaction of the two substrates in the absence of template under identical conditions. Figures 1 and 3 contain the data; the slopes of the lines are the approximate initial rates.[23,24]

Template 9 (Scheme III). Template **9** and 1 equiv of both amine (**10**) and bromomethylene (**11**) substrates were weighed accurately into an NMR tube such that addition of 0.500 mL of $CDCl_3$ (containing *sym*-tetrachloroethane, the internal standard—see above) gave a solution of 0.0040 M concentration of all partners. The solvent was added just prior

(36) The values determined for binding constants appeared insensitive to trace impurities of water or acid (DCl): at the concentrations (Table I) under study, regardless of whether the $CDCl_3$ (Aldrich FT NMR grade) was from a freshly opened bottle or from a bottle that had already been open (and in routine use) for a week or two, no differences were observed.

(37) Available from Cricket Software, Malvern, PA 19355.

to the placement of the tube in the NMR probe and a delay of approximately 2-3 min was incurred to tune the *sym*-tetrachloroethane line width to an acceptable level (~1.0 Hz). Acquisitions were then started immediately (that the ratio of substrates was 1.0:1.0 was double-checked by integration). The data are given in Figure 1.

Characterization of Reaction Product Bis[[2-[7-(acetylamino)-1,8-naphthyridinyl]]methyl]amine (14). The precipitate from either the catalyzed or uncatalyzed reaction was filtered off and then partitioned between aqueous $NaHCO_3$ and CH_2Cl_2. The CH_2Cl_2 layer was separated, dried (Na_2SO_4), and evaporated to give **14** as a solid: mp 238-241 °C dec; ^1H NMR (DMSO-d_6) 2.17 (6 H, s), 4.15 (4 H, s), 7.76 (2 H, d, J = 8.3 Hz), 8.32-8.39 (6 H, m), 11.04 (2 H, s) (the $(CH_2)_2NH$ proton was not visible); exact mass calcd for $C_{22}H_{20}N_7O_2$ [M + H - 2H]$^+$ 414.1679, found 414.1674.

Template 41 (Scheme V). Template **41** was insoluble in $CDCl_3$ alone. However, a soluble form was obtained by dissolving, in a 1.0:1.0 ratio, amino substrate **10** and template **41** in dichloromethane/methanol (9:1) and evaporating the solvent, with any residual traces of solvent being removed on a high-vacuum pump (complete removal of the solvent was established by ^1H NMR of the complex). The **10·41** complex thereby obtained could then be weighed accurately into an NMR tube and dissolved directly in $CDCl_3$ (0.500 mL) to give a 0.0040 M solution of template **41** and amino substrate **10**. After addition of the internal standard, the bromomethylene substrate **42** (1.0 equiv) was added just prior to placing the NMR tube in the probe, and acquisition, as with template **9**, was started immediately following tuning of the *sym*-tetrachloroethane line width. The data are given in Figure 3.

The experiment demonstrating inhibition of the **41**-catalyzed reaction between **10** and **42** was conducted as above, except that 1 equiv of **26** was added to the NMR tube (exchange is rapid) prior to the addition of **42**.

Characterization of Reaction Product *N*-[[2-[7-(Acetylamino)-1,8-naphthyridinyl]]methyl]-*N*-[[2-[7-oxo-1,8-naphthyridinyl]]methyl]amine (45). The precipitate from either the catalyzed or uncatalyzed reaction was filtered off and washed with a small amount of $CDCl_3$ to give **45**·HBr as a beige solid: mp 177-179 °C dec; ^1H NMR (DMSO-d_6) δ 2.20 (3 H, s), 4.30 (2 H, s), 4.45 (2 H, s), 6.59 (1 H, d, J = 9.3 Hz), 7.40 (1 H, d, J = 7.9 Hz), 7.56 (1 H, d, J = 8.2 Hz), 7.95 (1 H, d, J = 9.3 Hz), 8.18 (1 H, d, J = 7.9 Hz), 8.40 (1 H, d, J = 8.9 Hz), 8.44 (1 H, d, J = 8.2 Hz), 8.47 (1 H, d, J = 8.9 Hz), 10.99 (1 H, br s), 12.12 (1 H, br s); mass spectrum (FAB + NBA) 375 [**45** + H]$^+$.

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Stereocontrol during the Alkylation of Enolates Attached to π-Allyl-Mo(CO)$_2$Cp Systems

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Abstract: The preparations of dicarbonyl(η^5-cyclopentadienyl)(1-3-η-5-oxocyclohexenyl)molybdenum (**4**) and dicarbonyl(η^5-cyclopentadienyl)(1-3-η-5-oxocycloheptenyl)molybdenum (**27**) are described. Deprotonation of **4** using lithium diisopropylamide at -100 °C, followed by treatment of the enolate with electrophiles (alkyl halides, benzaldehyde, Michael acceptors), leads to stereospecific alkylation at C-4 anti to the Mo(CO)$_2$Cp group. Deprotonation of the alkylation products occurs regiospecifically at C-6 and enolate alkylation gives 4-exo,6-exo-disubstituted complexes stereospecifically. The corresponding seven-membered ring complex **27** is deprotonated regiospecifically at C-4 on treatment with base, and the enolate can be alkylated stereospecifically anti to the metal. The stereochemical outcome of nucleophile addition to the ketone of the alkylation products from **4** and **27** is different and is explained on the basis of conformational arguments. The conformation of the cycloheptenyl complexes **25** and **31a** were confirmed by single-crystal X-ray structure determination. $C_{14}H_{16}O_3Mo$ (**25**) crystallizes with space-group symmetry of $P2_1/c$. The unit-cell dimensions were a 11.694 (4), b 17.775 (6), c 13.114 (4) Å, β 96.38 (3)°, V 2708.9 (15) Å^3, and Z = 8. The structure was refined to convergence with a final value of R = 4.28%, R_w = 6.38% ($F \geq 6.0\sigma$). Similarly, $C_6H_{20}O_3Mo$ (**31a**) crystallized with space-group symmetry of $P2_1/c$. The unit cell dimensions were a 9.719 (3), b 12.955 (4), c 12.120 (4) Å, β 103.48 (2)°, V 1484.1 (8) Å^3, and Z = 4. This structure was refined to final values of R = 2.77%, R_w = 5.13% ($F \geq 6.0\sigma$).

One of our major interests is the use of electrophilic transition-metal π-complexes in stereocontrolled carbon-carbon bond formation.[1] This is illustrated schematically in Figure 1, where sequential nucleophile addition/hydride-abstraction/nucleophile addition reactions are used to introduce two carbon substituents with defined relative stereochemistry onto six- and seven-membered rings with use of reactive diene-Mo(CO)$_2$Cp complexes. We have recently begun to investigate the reactions of carbanions generated on π-allyl-molybdenum complexes; our earlier studies were aimed at using cyano-stabilized carbanions to generate quaternary carbon centers.[2] During these studies it was noted that there is an apparent stabilization of carbanion by the adjacent π-allyl-Mo(CO)$_2$Cp moiety, a fairly common occurrence in organometallic chemistry.[3] In the light of these experiments, and

(1) (a) Organoiron complexes: Pearson, A. J.; Kole, S. L.; Ray, T. *J. Am. Chem. Soc.* **1984**, *106*, 6060. Pearson, A. J.; Ray, T. *Tetrahedron Lett.* **1986**, *27*, 3111. Pearson, A. J.; Lai, Y. S.; Lu, W.; Pinkerton, A. A. *J. Org. Chem.* **1989**, *54*, 3882. (b) Organomolybdenum complexes: Pearson, A. J.; Khan, M. N. I.; Clardy, J. C.; Cun-heng, H. *J. Am. Chem. Soc.* **1985**, *107*, 2748. Pearson, A. J.; Khan, M. N. I. *J. Org. Chem.* **1985**, *50*, 5276.

(2) Pearson, A. J.; Khetani, V. D. *J. Am. Chem. Soc.* **1989**, *111*, 6778. See also ref 8 for acyclic systems.

(3) See, for example: Kundig, E. P. *Pure Appl. Chem.* **1985**, *57*, 1855. Williams, G. M.; Rudisill, D. E. *J. Am. Chem. Soc.* **1985**, *107*, 3357. Brookhart, M.; Rush, P. K.; Noh, S. K. *Organometallics* **1986**, *5*, 1745. Brookhart, M.; Nohn, S. K.; Timmers, F. J. *Organometallics* **1987**, *6*, 1829. Brookhart, M.; Noh, S. K.; Timmers, F. J.; Hong, Y. H. *Organometallics* **1988**, *7*, 2458. Davies, S. G. *Organotransition Metal Chemistry: Applications to Organic Synthesis*; Pergamon Press: New York, 1982; Chapter 5.

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