Artificial Redox Enzymes. 1. Synthetic Strategies

Ding Rong, Hongping Ye, Todd R. Boehlow, and Valerian T. D'Souza*

Department of Chemistry, University of Missouri—St. Louis, St. Louis, Missouri 63121

Received July 26, 1991

Organic models of flavoenzymes consist of a binding site covalently attached to a flavin derivative acting as the catalytic site. The earlier reported synthesis of such a model using α-cyclodextrin as the binding site proved to be difficult to reproduce with β-cyclodextrin. The synthetic strategy involved attaching a fully constructed riboflavin onto a cyclodextrin by a nucleophilic reaction. Riboflavin was found to decompose under the reaction conditions. A new method for the synthesis of flavocyclodextrins involving construction of the flavin moiety onto cyclodextrin is convenient and can be used to synthesize 6-flavocyclodextrins and 2-flavocyclodextrins.

Flavoenzymes are important classes of enzymes which catalyze a variety of reactions including oxidation, hydroxylation, dehydrogenation, etc.[1] They essentially are comprised of a riboflavin molecule bound noncovalently to a substrate binding site. The substrate binds to the binding site and then undergoes a redox reaction catalyzed by riboflavin. The models of flavoenzymes, consisting of a flavin molecule which acts as a catalyst, attached covalently to a binding site,[2] can facilitate the study of the mechanism of action of these enzymes and be useful catalysts for a variety of chemical transformations.

Important models of flavoenzymes that have been reported in the literature include flavopapain,[3] antibody–flavin complex,[4] flavo-crown ether,[5] flavinophane,[6] and flavocyclodextrin[7] [6-(8α-*S*-riboflavo)-α-cyclodextrin (18)]. Flavopapain and antibody–flavin complexes consist of a riboflavin molecule attached to a protein acting as the binding site. Although these models made useful contributions in showing that proteins can be manipulated, they have the same limitations of complexity and instability as real enzymes. A flavo-crown ether consists of a flavin attached to a crown ether and does not have all these limitations, but it can bind only substrates that possess a positive charge. Catalytic processes in flavinophanes have not yet been demonstrated. Flavocyclodextrins show the best promise as chemically useful models of flavoenzymes because cyclodextrins have been shown to possess enzyme-like binding capability for small organic molecules,[8] and 18 synthesized by Tabushi[7] is reported to be efficient in electron-transfer and redox reactions. However, this study was limited to one flavocyclodextrin where flavin was attached to the primary side of α-cyclodextrin, and only electron-transfer reactions of this model from dihydronicotinamides were investigated.[9]

A series of flavocyclodextrins and a thorough evaluation of these models, especially those having flavin attached to the secondary side of cyclodextrin, would lead to a better understanding of the real enzymes. Further, our attempts to synthesize the β-cyclodextrin analogue of the reported model failed, and thus the reported procedure is not general. β-Cyclodextrin is perhaps the most widely studied of the three (α-, β-, and γ-) cyclodextrins. We now report the synthesis of two new β-flavocyclodextrins prepared by a strategy which is more general in nature and superior in terms of yield.

Scheme I

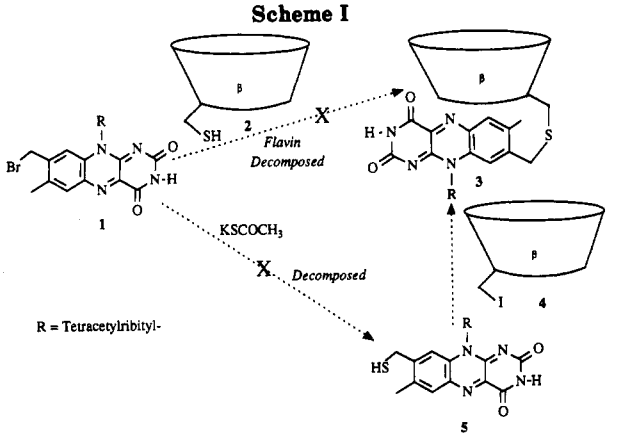


Table I. Attempted Coupling of Riboflavin with β-Cyclodextrin by Conventional Methods[a]

	pH	solvent	time	results
a	6.0	H_2O, DMF	3 d	no reaction
b	7.5	Tis-HCl buffer, DMSO	25 h	no reaction
c	–	pyridine	15 min	flavin decompose, no reaction for **2**
d	10	H_2O–$NaHCO_3$	12 h	flavin decompose, no reaction for **2**

[a] Reactants, **1** and **2**; temperature, 25 °C.

Results and Discussion

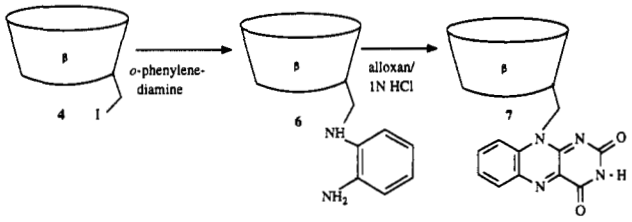
Two strategies for the synthesis of organic models of flavoenzymes are known: (1) coupling of functionalized flavins onto a binding site, the strategy used for synthesis of flavopapain[3] and flavocyclodextrin;[7] (2) building a flavin moiety onto a binding site, the scheme used for the synthesis of flavo-crown ether.[5]

The first strategy would appear to be more attractive for the synthesis of flavocyclodextrins because (1) the synthesis of flavin derivatives as electrophiles[10] and cyclodextrin derivatives as nucleophiles[11] are well developed, and this strategy is the conventional method for synthesizing cyclodextrin derivatives; (2) the flavin moiety is attached to cyclodextrin in the final step of the reaction sequence and the cyclodextrin derivatives are isolated by Sephadex chromatography. This would be more econom-

(1) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, 1979; Chapters 10, 11, 12.
(2) D'Souza, V. T.; Bender, M. L. *Acc. Chem. Res.* **1987**, *20*, 146.
(3) Levine, H. L.; Kaiser, E. T. *J. Am. Chem. Soc.* **1978**, *100*, 7670.
(4) Schultz, P. *Acc. Chem. Res.* **1989**, *22*, 287.
(5) Shinkai, S.; Ishikawa, Y.; Shinkai, H.; Tsuno, T.; Makishima, H.; Ueda, K.; Manabe, O. *J. Am. Chem. Soc.* **1984**, *106*, 1801.
(6) Seward, E. M.; Hopkins, R. B.; Sauerer, W.; Tam, S.; Diederich, F. *J. Am. Chem. Soc.* **1990**, *112*, 1783.
(7) Tabushi, I.; Kodera, M. *J. Am. Chem. Soc.* **1987**, *109*, 4734.
(8) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978.
(9) Professor Tabushi passed away on March 22, 1987, and this work has been discontinued.
(10) Narasimhan, K.; Wingard, L. B. *J. Mol. Catal.* **1986**, *34*, 253.
(11) Fugita, K.; Ueda, T.; Imoto, T.; Tabushi, I.; Toh, N.; Koga, T. *Bioorg. Chem.* **1982**, *11*, 73.

0022-3263/92/1957-0163$03.00/0

Scheme II



ical in terms of time and yield since the second strategy would involve similar purifications for each of the steps in the reaction scheme.

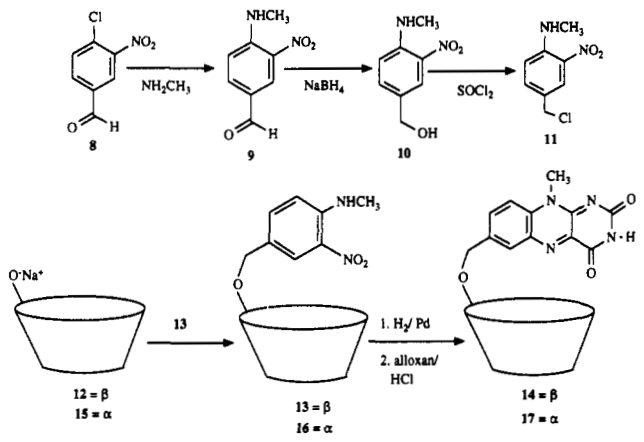
(a) Attachment of Flavin to the Primary Side of β-Cyclodextrin with the Conventional Method (Scheme I). This method essentially involves attaching a derivative of commercially available riboflavin to cyclodextrin by a nucleophilic substitution reaction. Synthesis of 6-(8α-*S*-riboflavo)-β-cyclodextrin (**3**) was attempted using the published procedure[7] for **18**. As shown in Table I, reactions of 6-mercapto-β-cyclodextrin[11] (**2**) with 8α-bromo-2′,3′,4′,5′-tetraacetylriboflavin[10] (**1**) under variety of conditions were all unsuccessful.

Attempts to synthesize **3** by a nucleophilic attack of a riboflavin derivative 8α-mercapto-2′,3′,4′,5′-tetraacetylriboflavin (**5**) on 6-iodo-β-cyclodextrin[12] (**4**) were made. However, the preparation of **5** by the reaction of **1** with potassium thioacetate and subsequent hydrolysis failed. **1** decomposed at the initial stages under the reaction conditions, and the product could not be isolated. Riboflavin derivatives were found to decompose rapidly even under mildly basic conditions, and thus any synthetic manipulation of riboflavin involving basic conditions is difficult. The strategy to build flavocyclodextrins by attaching fully constructed flavins onto cyclodextrins by a substitution reaction is not facile.[13]

(b) A New Method for Attaching Flavin to the Primary Side of Cyclodextrin. In contrast to the conventional method described above, the new approach involves synthesizing an *o*-phenylenediamine derivative of cyclodextrin and then condensing it with alloxan to give the desired flavocyclodextrin. However, in the synthesis of flavins, the condensations of alloxan with *o*-phenylenediamine derivatives are carried out at high temperature[14] in the presence of acids such as sulfuric acid, hydrochloric acid, acetic acid, and boric acid.[15] Since cyclodextrins hydrolyze under acidic conditions at high temperature, this approach can be successful only if the *o*-phenylenediamine derivative of cyclodextrin can be condensed with alloxan under conditions mild enough to minimize acid-induced decomposition of cyclodextrin. Approximately 1% of the ring of β-cyclodextrin will be hydrolyzed in 1.15 N HCl at 60 °C in 30 min,[16] and assuming that the rate of cleavage of monofunctionalized β-cyclodextrin is the same as that of the β-cyclodextrin, the amount of cyclodextrin hydrolyzed under these conditions can be tolerated in a reaction sequence.

Via Scheme II, the reaction of **4** with a large excess of *o*-phenylenediamine gave 6-(2-aminoanilino)-β-cyclodextrin (**6**). Upon workup, the TLC showed a single spot which had UV absorbance and charred upon H_2SO_4/heat treatment, indicating the presence of both the phenylenediamine moiety as well as the cyclodextrin moiety in the product. ^1H and ^{13}C NMR spectra indicated that one *o*-phenylenediamine molecule was attached to the 6-position of β-cyclodextrin. The ^1H NMR spectrum showed all the peaks of β-cyclodextrin and aromatic multiplets due to the *o*-phenylenediamine moiety in the range between 6.3 and 6.6 ppm. The ^{13}C NMR peaks at 45.3, 70.0, and 84.5 ppm for C′6, C′5, and C′4 of the substituted glucose unit are shifted 15.1 and 2.0 ppm upfield and 2.8 ppm downfield, respectively, from the original peaks for C6, C5, and C4 of β-cyclodextrin, indicating that the substituent is at the 6-position.[17] However, the color of an aqueous solution of **6** turns dark brown when exposed to air for 1 day, suggesting the oxidation of the *o*-phenylenediamine moiety, and the material could not be characterized by elemental analysis. Therefore, freshly prepared product was placed under vacuum for 1 h at room temperature and then used immediately in the following reaction to avoid such oxidation.

Scheme III



A mixture of **6** with alloxan monohydrate was dissolved in aqueous 1 N HCl and heated in a refluxing acetone bath[18] for 15 min. Reverse-phase chromatography using C18 column and 10% aqueous acetonitrile as the eluent gave pure 6-(10-*N*-isoalloxazinomethyl)-β-cyclodextrin (**7**) as indicated by a single yellow TLC spot which charred on H_2SO_4/heat treatment and confirmed by ^1H and ^{13}C NMR and elemental analyses.

(c) Attachment of Flavin to the Secondary Side of Cyclodextrins. Binding studies have shown that the secondary side of cyclodextrin is more important than the primary side in enzyme mimic chemistry because the substrates generally bind to cyclodextrins with their functional groups oriented toward this side.[19] Thus, catalytic groups attached to the secondary side of cyclodextrins should be more effective enzyme mimics. Conventional methods to modify this side of cyclodextrin give a low yield of the product[20] and distort the cyclodextrin cavity.[21] Our approach for building organic models of flavoenzymes illustrated here by the synthesis of 2-[(7α-*O*-10-methyl-7-isoalloxazino)methyl]-β-cyclodextrin (**14**) (Scheme III) overcomes these problems.

4-Chloro-3-nitrobenzaldehyde (**8**) was reacted with methylamine to yield 4-(methylamino)-3-nitrobenzaldehyde (**9**), which was then reduced by $NaBH_4$ to 4-(methyl-

(12) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Tetrahedron* **1987**, *43*, 1571.
(13) Footnote 8 in ref 7.
(14) Ghisla, S.; Mayhew, S. G. *Eur. J. Biochem.* **1976**, *63*, 373.
(15) Lambooy, P. *Heterocycl. Compd.* **1967**, *9*, 118.
(16) Szejtli, J.; Budai, Z. *Acta Chim. Acad. Sci. Hung.* **1976**, *91*, 73.
(17) Ueno, A.; Breslow, R. *Tetrahedron Lett.* **1982**, *23*, 3451.
(18) It was important to control the temperature of the reaction using a refluxing acetone bath because use of an oil bath with a fluctuating temperature resulted in either incomplete reaction or the decomposition of the cyclodextrin ring.
(19) VanEtten, R. C.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *69*, 3242.
(20) Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390.
(21) Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275.

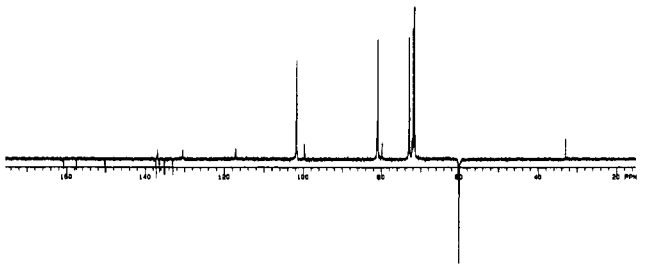


Figure 1. INEPT ^{13}C NMR spectrum of **14** in D_2O.

amino)-3-nitrobenzenemethanol (**10**). Reaction of thionyl chloride with **10** afforded 4-(chloromethyl)-*N*-methyl-2-nitrobenzenamine (**11**). **9**, **10**, and **11** were characterized by their ^1H NMR spectra and elemental analysis. Although **11** recrystallized from ether in the presence of a large excess of HCl, it is not the hydrochloride salt of the secondary amine as evidenced by elemental analysis, indicating that it is a weak base.[22] **11** was used as an electrophile to react with sodium β-cyclodextrin alkoxide **12** to yield 2-[4-(methylamino)-3-nitrobenzyl]-β-cyclodextrin (**13**). The synthesis of **13** from **11** and **12** represents a new method for selective monofunctionalization of the 2-position of cyclodextrins, and a full discussion of this method and the structure assignment of **13** based on ^{13}C NMR have been reported.[21] Due to the previous controversies regarding the position of the substituent on cyclodextrins (primary vs secondary) when it reacts with electrophiles in the presence of various bases,[23] an INEPT ^{13}C NMR spectrum was examined to confirm the ^{13}C NMR assignments published previously.[21] In INEPT ^{13}C NMR spectroscopy, the positive peak at 77.9 ppm assigned to C′2 and the absence of any negative peaks corresponding to C6′ of the substituted glucose unit further support the assumption that the *o*-phenylenediamine moiety is attached to the hydroxyl group at the 2-position of the cyclodextrin. **11** was also reacted with α-cyclodextrin alkoxide **15** using the method described above to yield 2-[4-(methylamino)-3-nitrobenzyl]-α-cyclodextrin (**16**). The structure of **16** was elucidated by ^1H, ^{13}C, and INEPT ^{13}C NMR spectra as described for the characterization of **13**. This clearly indicates that the above-described method is general and can be used for attaching substituents onto the secondary side of cyclodextrins without the stereochemical inversions and the ring distortion caused by the conventional methods for modification of the secondary side of cyclodextrin.[21]

13 was hydrogenated and then condensed with alloxan monohydrate to yield **14**. The structure of **14** was elucidated by ^1H and ^{13}C NMR. The ^1H NMR spectrum in D_2O exhibits all the normal peaks for β-cyclodextrin in the range of 4.4–3.0 ppm; peaks in the range of 7.2–7.45 ppm can be assigned to aromatic protons of the flavin moiety by comparison with the spectrum of flavins.[24] The absence of any resonance at >7.5 ppm in the ^1H NMR in D_2O and presence of a broad peak at 11.41 ppm in the ^1H NMR in DMSO-d_6 indicate the presence of the acidic N-H(3) in this compound. In ^{13}C NMR (D_2O) the 10 peaks at >116 ppm can be assigned to the aromatic carbons of the flavin moiety in comparison to the spectrum of flavins.[25] These assignments are further supported by INEPT ^{13}C NMR in D_2O (Figure 1) which shows three positive peaks corresponding to three methine carbons and seven negative peaks corresponding to quaternary carbons in the structure of the flavin moiety. The six normal peaks for the β-cyclodextrin moiety in the ^{13}C NMR in D_2O of **14** can be assigned[26] as 60.1 (C6), 71.6 (C2), 71.9 (C5), 72.9 (C3), 80.93 (C4), and 101.6 (C1). The peak at 33.0 can be assigned to the CH_3 group because its chemical shift falls in the expected range and its appearance as a positive peak on INEPT ^{13}C NMR spectrum. The peaks at 79.8 and 99.6 ppm can be assigned to C′2 and C′1, respectively, of the substituted glucose unit because the flavin group, like the tosyl group, is an electron-withdrawing group and when attached to the hydroxyl group at the 2-position of β-cyclodextrin, it will cause a large downfield chemical shift on C′2 and a significant upfield chemical shifts on C′1 of the substituted glucose unit.[17] These two peaks are shown as positive peaks in INEPT ^{13}C NMR (D_2O), indicating that they correspond to methine carbons.

Hydrogenation of **16** followed by condensation with alloxan afforded 2-[(7α-*O*-10-methyl-7-isoalloxazino)-methyl]-α-cyclodextrin (**17**). The structure assignment for **17** was made in a manner similar to the one described above for **14**. Thus, Scheme III is general and can be used to build 2-flavocyclodextrins. We are now in the process of using this scheme to vary the length and the position of the linker between cyclodextrins and the flavin moiety.

Conclusion

A general method for the synthesis of flavocyclodextrins with flavins attached to either the primary or secondary side of cyclodextrin is now available. The evaluation of these redox enzyme mimics in terms of their tertiary structures and catalytic properties is in progress and will be the subject of another paper.

Experimental Section

General Method. The chemical shifts (δ) are reported downfield from TMS with $CHCl_3$ and DMSO-d_5 as internal references for $CDCl_3$ and DMSO-d_6 and TMS as an external reference for D_2O. TLC was performed on aluminum sheets precoated with 0.2-mm silica 60 F_{254} (E. Merck, Germany). For cyclodextrin derivatives, the eluent for TLC was 1-butanol–ethanol–water, 5:4:3 by volume, and the spots were detected first by UV lamp if the product contained a chromophore and then by charring with heat after spraying the plate with 50% methanolic sulfuric acid. Gel filtrations were performed on a Sephadex (G-25-100, Sigma Chemical Co.) column (length 95 cm, i.d. 28 cm) using distilled water as eluent. HPLC was performed on a Kromasil ^{18}C, 5 μm, length, 250 mm, i.d., 10 mm column from Alltech; solvent, acetonitrile–distilled water, 1:9 by volume; flow rate, 3 mL/min. α-Cyclodextrin and β-cyclodextrin (generous gift from AMAIZO) were dried overnight in a drying piston in refluxing 1-butanol under vacuum. Riboflavin and **8** were purchased from Aldrich Chemical Co. DMF and DMSO were dried over CaH_2 for overnight. **2**,[11] **1**,[10] and **4**[12] were synthesized according to literature procedures and had physical and spectral properties identical to the ones reported. The samples were dried at room temperature under high vacuum for several days before submitting them to elemental analysis by Atlantic Microlab, Inc., Atlanta, GA.

The Reaction of 2 with 1. **2** was reacted with **1** using the following procedures: (a) To a solution of **2** (100 mg, 0.087 mmol) in distilled water (5 mL) and DMF (5 mL) was added **1** (30 mg, 0.047 mmol), and the mixture was allowed to stand at room temperature for 3 days. The reaction did not occur as indicated

(22) The absence of polymerization of **11** by intermolecular condensation of the secondary amine and the benzyl chloride in the molecule can be attributed to the weakly basic nature of the amine caused by conjugation of the amine with the phenyl ring and the electron-withdrawing effect of the nitro group at the ortho position.

(23) Takahashi, K.; Hatori, K.; Toda, F. *Tetrahedron Lett.* **1984**, *25*, 3331.

(24) Grande, H. J.; van Schagen, C. G.; Jarbandhan, T.; Muller, F. *Helv. Chim. Acta* **1977**, *60*, 348.

(25) Grande, H. J.; Gast, R.; van Schagen, C. G.; van Berkel, W. J. *Helv. Chim. Acta* **1977**, *60*, 367.

(26) Inoue, Y. *JEOL News* **1987**, *23A*, 36.

by TLC and ^{13}C NMR of the precipitate obtained by addition of acetone (100 mL) to the reaction mixture and filtration. (b) To a solution of **2** (40 mg, 0.035 mmol) in DMSO (5 mL) and Tris-HCl buffer (5 mL, 0.1 M, pH 7.5) was added **1** (10 mg, 0.016 mmol), and the mixture was allowed to stand at room temperature for overnight. The reaction did not occur as indicated by TLC. (c) To a solution of **2** (63 mg, 0.055 mmol) in pyridine (10 mL) was added **1** (23 mg, 0.036 mmol), and the mixture was allowed to stand at room temperature for 15 min. Although TLC data indicated that some reaction had taken place, ^1H and ^{13}C NMR spectra of the precipitate obtained by addition of acetone (150 mL) and filtration showed that it was the recovered β-cyclodextrin. (d) To a solution of **2** (50 mg, 0.043 mmol) and ammonium bicarbonate (200 mg, 2.5 mmol) in distilled water (5 mL) was added **1** (20 mg, 0.031 mmol), and the mixture was stirred at room temperature for 1 h. TLC of the reaction mixture gave four yellow spots, none of which could be charred by H_2SO_4/heat treatment. The yellow compounds were isolated by preparative TLC (20 × 20 cm, 1000 μm silica gel, (1-butanol–ethanol–water, 5:4:3 by volume). ^1H NMR showed no peaks for the β-cyclodextrin moiety and did not match with that of **1**.

Reaction of 1 with Potassium Thioacetate. To a solution of **1** (50 mg, 0.078 mmol) in DMF (5 mL) was added potassium thioacetate (10 mg, 0.088 mmol), and the mixture was allowed to stand at room temperature for 1 h. TLC showed same pattern as for the reaction of **2** with **1** (experiments c and d), indicating decomposition of the flavin molecule.

6-(10-*N*-Isoalloxazinomethyl)-β-cyclodextrin (7). To a solution of **4** (2.2 g, 1.8 mmol) in DMF (30 mL) was added *o*-phenylenediamine (4.4 g, 41 mmol) in DMF under nitrogen (5 mL) at 115 °C while stirring, and the mixture was allowed to stand under the same condition for 3 h. The solution was then cooled to room temperature, and acetone (400 mL) was added to this solution to generate a white precipitate. The precipitate was collected by filtration, redissolved in water (40 mL), and extracted three times with 50-mL portions of chloroform. Acetone (300 mL) was added to the aqueous layer to give a precipitate, which upon filtration gave **6** (2.1 g, 94%) as a light brown solid. The solid turned dark brown on exposure to air, and purification to give an analytical sample was not possible. A freshly prepared sample was found to be suitable for condensation with alloxan in the next step. The ^{13}C NMR spectrum showed all the normal peaks of β-cyclodextrin at 60.4, 72.0, 72.5, 73.5, 81.7 and 102.4 ppm; the peaks for the substituted glucose of cyclodextrin at 45.3, 70.0, and 84.5 ppm; and the six peaks for the *o*-phenylenediamino moiety at 111.1, 114.8, 117.6, 118.1, 135.6, and 136.3 ppm.

To a solution of alloxan monohydrate (1.0 g) in dilute hydrochloridic acid (2 mL of HCl and 18 mL of water) at 78 °C was added a solution of freshly prepared **6** (500 mg) in water (30 mL), and the reaction mixture was kept at 78 °C for 15 min. Acetone (500 mL) was added to the cooled mixture, and the precipitate thus obtained was filtered and dried under vacuum at room temperature to afford a yellow powder which was further purified by HPLC. The yellow fraction which eluted at 6.4 min was evaporated to dryness, washed with acetone (10 mL), and dried under vacuum at room temperature to afford **7** (0.155 g, 25%) as a yellow powder: R_f 0.22, yellow and charred by H_2SO_4/heat treatment; ^1H NMR (DMSO-d_6) δ 11.24 (1 H, s, NH(3)), 8.2–7.5 (4 H, m, aromatic protons), 6.1–3.0 (protons of cyclodextrin moiety); ^{13}C NMR (DMSO-d_6) δ 159.7, 155.5, 150.7, 138.6, 134.8, 134.3, 134.1, 131.4, 125.9, 117.8 (carbons of flavin), 102.0 (C1), 84.6, 81.8 (C4), 73.0 (C3), 72.6 (C5), 72.1 (C2), 67.0, 60.2 (C6), 46.5 (C6′); INEPT ^{13}C NMR (DMSO-d_6) δ 159.7, 155.5, 150.7, 138.6, 134.8, 134.1, 60.2, 46.5 (negative peaks for C and CH_2) and the rest of the peaks are positive for CH and CH_3; UV–vis (H_2O) λ_{max} 265 (ε 2.71 × 10^4), 342 (ε 6.61 × 10^3), and 435 nm (ε 7.54 × 10^3). Anal. Calcd for $C_{52}H_{74}N_4O_{36}\cdot 7H_2O$: C, 42.86; H, 6.09; N, 3.85. Found: C, 42.86; H, 6.06; N, 3.75.

4-(Methylamino)-3-nitrobenzaldehyde (9). To a solution of **8** (4.0 g, 22 mmol) in ethanol (95%, 30 mL) was added methylamine solution (40%, 50 mL), and the mixture was refluxed for 3 h. The mixture was stored overnight at about 5 °C to give orange crystals identified by ^1H NMR as the Schiff base of the aldehyde of **8**. These crystals were filtered, washed with water, dissolved in 400 mL of 1 N HCl, and stirred overnight to afford a yellow precipitate. The mixture was filtered and washed with water to yield 3.2 g (81%) of **9** as a yellow powder: mp 173.5–175.0 °C, (lit.[27] mp 171–172 °C); ^1H NMR ($CDCl_3$) δ 9.78 (1 H, s, CH=O), 8.64 (1 H, d, $J_{2,6}$ = 1.9 Hz, H-2), 8.54 (1 H, broad, NH), 7.97 (1 H, dd, $J_{5,6}$ = 9 Hz, $J_{2,6}$ = 1.5 Hz, H-6), 6.94 (1 H, d, $J_{5,6}$ = 9 Hz, H-5), 3.11 (3 H, d, $J_{1',2'}$ = 5.2 Hz, CH_3-2′). Anal. Calcd for $C_8H_8N_2O_3$: C, 53.33; H, 4.48; N, 15.55. Found: C, 53.45; H, 4.55; N, 15.58.

4-(Methylamino)-3-nitrobenzenemethanol (10). Sodium borohydride (0.32 g, 8.5 mmol) was added to a solution of **9** (3.0 g, 16.6 mmol) in ethyl alcohol (100%, 20 mL), and the mixture was stirred at room temperature for 3 h. The reaction mixture was cooled in an ice bath, and 8.5 mL of 2 N HCl was added dropwise. The solution was adjusted to pH 10 by addition of proximately 10 mL of concentrated ammonium hydroxide. The solution was extracted three times with 60-mL portions of chloroform, and the combined organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was recrystallized from water to afford 1.7 g (56%) of **10** as an orange solid: mp 126.0–127.0 °C (lit.[27] mp 114–115 °C); ^1H NMR (DMSO-d_6) δ 8.11 (1 H, d, $J_{1',2'}$ = 5.1 Hz, NH-1′), 7.98 (1 H, d, $J_{2,6}$ = 1.8 Hz, H-2), 7.45 (1 H, dd, $J_{5,6}$ = 8.8 Hz, $J_{2,6}$ = 1.7 Hz, H-6), 6.89 (1 H, d, $J_{5,6}$ = 8.8 Hz, H-5), 5.22 (1 H, t, $J_{3',4'}$ = 5.7 Hz, OH-4′), 4.39 (2 H, d, $J_{3',4'}$ = 5.6 Hz, CH_2-3′), 2.94 (3 H, d, $J_{1',2'}$ = 5.1 Hz, CH_3-2′); ^{13}C NMR (DMSO-d_6) δ 145.0, 135.50, 130.20, 129.16, 123.36, 113.95, 61.78 (CH_2), 29.65 (CH_3). Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 53.02; H, 5.52; N, 15.48.

4-(Chloromethyl)-*N*-methyl-2-nitrobenzenamine (11). **10** (1.0 g, 5.5 mmol) was added to a solution of thionyl chloride (25 mL) cooled in a dry ice–acetone bath to below −60 °C. The dry ice–acetone bath was removed after **10** had completely dissolved, and the reaction mixture was allowed to warm up to room temperature and kept at room temperature for an additional 1 h. The reaction mixture was evaporated at room temperature under reduced pressure to almost dryness, and the residue was recrystallized from diethyl ether to afford 0.6 g (55%) of **11** as a yellow solid: mp 126–128 °C; ^1H NMR (DMSO-d_6) δ 8.29 (1 H, broad, NH), 8.15 (1 H, d, $J_{3,5}$ = 2.2 Hz, H-3), 7.60 (1 H, dd, $J_{5,6}$ = 9.0 Hz, $J_{3,5}$ = 2.2 Hz, H-5), 7.01 (1 H, d, $J_{5,6}$ = 9.0 Hz, H-6), 4.76 (2 H, s, CH_2), 2.95 (3 H, s, CH_3). Anal. Calcd for $C_8H_9ClN_2O_2$: C, 47.89; H, 4.52; Cl, 17.67; N, 14.00. Found: C, 47.78; H, 4.49; Cl, 17.78; N, 13.82.

2-[4-(Methylamino)-3-nitrobenzyl]-β-cyclodextrin (13). To a solution of β-cyclodextrin (1.0 g, 0.88 mmol) in DMF (40 mL) was added sodium hydride (35 mg, 60% in oil, 0.88 mmol), and the mixture was stirred overnight until the solution became clear. This solution was added dropwise to a solution of **11** (0.173 g, 0.88 mol) in DMF (5 mL), and the mixture was allowed to stand at room temperature for 30 min. β-Cyclodextrin and its derivatives were precipitated out by the addition of acetone (500 mL). The precipitate was filtered and washed with acetone (100 mL) to give 1.0 g of crude product containing only **13** and β-cyclodextrin as indicated by TLC. The mixture was purified by Sephadex chromatography to furnish 0.40 g (35%) of **13** as yellow powder: R_f 0.55; ^1H NMR (DMSO-d_6) δ 8.21 (1 H, d, J = 4.7 Hz, N-H), 8.07 (1 H, s, H-2), 7.60 (1 H, d, $J_{5,6}$ = 9.0 Hz, H-6), 7.01 (1 H, d, $J_{5,6}$ = 9.0 Hz, H-5), 6.0–3.2 (proton of β-cyclodextrin), 2.96 (3 H, d, J = 4.7 Hz, CH_3); ^1H NMR (D_2O) δ 7.92 (1 H, s, H-2), 7.62 (1 H, d, $J_{5,6}$ = 8.7 Hz, H-6), 6.90 (1 H, d, $J_{5,6}$ = 9.0 Hz, H-5), 5.13–4.85 (7 H, m, H1), 3.93–3.30 (42 H, m, H2, H3, H4, H5, H6), 2.97 (3 H, s, CH_3); ^{13}C NMR (D_2O) δ 146.3, 136.0, 129.8, 125.5, 123.8, 114.5, 101.8 (C1), 100.4 (C1′), 81.8 (C4′), 80.9 (C4), 77.9 (C2′), 73.2–71.8 (C2,3,5), 59.8 (C6), 29.5 CH_3); INEPT ^{13}C NMR (D2O) δ (negative peaks for C and CH_2) 146.3, 129.8, 123.8, 59.8; δ (positive peaks for CH and CH_3) rest of the peaks shown in above ^{13}C NMR. Anal. Calcd for $C_{50}H_{78}N_2O_{37}\cdot 6H_2O$: C, 42.68; H, 6.45; N, 1.99. Found: C, 42.79; H, 6.45; N, 1.93.

2-[(7α-*O*-10-Methyl-7-isoalloxazino)methyl]-β-cyclodextrin (14). Crude **13** (0.46 g, containing 40% of pure **13**) dissolved in methanol (80 mL) was hydrogenated in the presence of Pd/C (0.2, 10%) for approximately 24 h to give a colorless solution. After the reaction mixture was filtered, the filtrate was evaporated under vacuum below 40 °C and acetone (100 mL) was

(27) Clark-Lewis, J. W.; Aldous, G. L.; Thomson, M. J. *Aust. J. Chem.* 1976, *29*, 2219.

added to the residue. The precipitate was filtered, washed with acetone (160 mL), and dried to provide a light brownish solid. This solid was added to a solution of alloxan monohydrate (0.5 g) in HCl (5 mL, 1 N) and heated in a refluxing acetone bath for 30 min. After the reaction mixture was cooled to room temperature, acetone (100 mL) was added to the reaction mixture to give a yellow precipitate which was filtered, washed with acetone, and purified by Sephadex chromatography to give two yellow fractions. The first yellow fraction between 250 and 320 mL was collected and concentrated to approximately 1 mL. Addition of acetone (100 mL) to this and filtration provided 30 mg (24%) of **14** as a yellow powder: mp >250 °C; R_f 0.28; ^{1}H NMR (DMSO-d_6) δ 11.41 (1 H, s, NH(3)), 8.2–7.9 (3 H, m, aromatic), 6.1–3.0 (protons of β-cyclodextrin moiety); ^{13}C NMR (DMSO-d_6) δ 160.2, 156.1, 151.5, 139.1, 136.5, 135.0, 134.7, 133.2, 130.5, 117.0 (carbons of flavin), 102.0 (C1), 100.02 (C'1), 81.60 (C4), 80.01 (C'2), 73.10 (C3), 72.41 (C5), 72.05 (C2), 60.0 (C6), 32.0 (CH_3); ^{1}H NMR (D_2O) δ 7.45–7.20 (3 H, aromatic protons), 4.35 (7 H, m, H1), 3.30–3.01 (28 H, m, H3, H6, H5), 3.01–2.75 (14 H, m, H2, H4); ^{13}C NMR (D_2O) δ 160.7, 157.4, 150.1, 137.2, 136.8, 136.4, 135.1, 133.0, 130.4, 117.1 (carbons of flavin), 101.6 (C1), 99.6 (C'1), 80.9 (C4), 79.8 (C'2), 72.9 (C3), 71.9 (C5), 71.6 (C2), 60.1 (C6), 33.0 (CH_3); INEPT ^{13}C NMR (D_2O) δ 136.8, 130.4, 117.1, 101.6, 99.6, 80.9, 79.8, 72.9, 71.9, 71.6, 33.0 (positive peaks for CH and CH_3), 160.7, 157.4, 150.1, 137.2, 136.4, 135.1, 133.0, 60.1 (negative peaks for C and CH_2); UV-vis (H_2O) λ_{max} 265 (ϵ 3.25 × 10^4), 342 (ϵ 7.24 × 10^3), and 435 nm (ϵ 8.40 × 10^3). Anal. Calcd for $C_{54}H_{78}N_4O_{37}\cdot 7H_2O$: C, 43.20; H, 6.18; N, 3.73. Found: C, 43.29; H, 6.17; N, 3.78.

2-[4-(Methylamino)-3-nitrobenzyl]-α-cyclodextrin (16). Sodium hydride (160 mg, 60% in oil, 4.0 mmol) was added to a solution containing α-cyclodextrin (3.9 g, 4.0 mmol) in a mixture of DMF (40 mL) and DMSO (40 mL), and the mixture was stirred for 5 h. To this mixture was added a DMF (5 mL) solution containing **11** (0.80 g, 4.0 mmol), and the resultant mixture was allowed to stand at room temperature for 2 h. At the end of this time, cyclodextrin and its derivatives were precipitated by addition of 1 L of acetone. The precipitate was collected and washed with acetone to give 4.0 g of crude products containing only **16** and unreacted α-cyclodextrin as indicated by TLC; 300 mg of this crude product was purified by Sephadex chromatography to provided 50 mg (15%) of **16** as an orange powder: R_f 0.56; ^{1}H NMR (D_2O) δ 8.07 (1 H, s), 7.57 (1 H, d, $J_{5,6}$ = 9.2 Hz), 6.93 (1 H, d, $J_{5,6}$ = 9.1 Hz), 5.04 (7 H, m, H1), 4.10–3.72 (28 H, m, H3, H6, H5), 3.70–3.48 (14 H, m, H2, H4), 2.98 (3 H, s, CH_3); ^{13}C NMR (D_2O) δ 147.8, 139.0, 131.5, 127.8, 125.3, 115.8 (for aromatic carbons), 102.2 (C1), 100.8 (C1'), 82.4 (C4), 80.1 (C2'), 74.3 (C3), 73.8, 73.4 (C5), 72.8 (C2), 61.5 (C6), 31.4 (CH_3); INEPT ^{13}C NMR (D_2O) δ (negative peaks for C and CH_2) 147.8, 131.5, 125.3, 73.8, 61.5, (positive peaks for CH or CH_3) the rest of the peaks shown in the above ^{13}C NMR. Anal. Calcd for $C_{44}H_{68}N_2O_{32}\cdot 5H_2O$: C, 43.05; H, 6.44; N, 2.28. Found: C, 43.32; H, 6.26.; N, 2.42.

2-[(7α-*O*-10-Methyl-7-isoalloxazino)methyl]-α-cyclodextrin (17). A solution of 1.5 g of crude **16** containing 17% of pure **16** in methanol (250 mL) was hydrogenated in the presence of Pd/C (5%, 0.3 g) at room temperature for approximately 24 h to give a colorless solution. After the mixture was filtered, the filtrate was evaporated under vacuum below 40 °C, and acetone (20 mL) was added to the residue, filtered, washed with acetone, and dried to afford 1.5 g of a light yellow solid; 0.70 g of the solid was added to a solution of alloxan monohydrate (2.8 g, 18 mmol) in HCl (10 mL, 1 N) and heated in a refluxing acetone bath for 40 min. After the reaction mixture was cooled to room temperature, acetone (200 mL) was added to give a yellow precipitate which was filtered, washed with acetone, and purified by Sephadex chromatography to afford 50 mg (40%) of **17** as a yellow powder: R_f 0.24; ^{1}H NMR (D_2O) δ 7.38 (1 H, d, $J_{8,9}$ = 8.8 Hz), 7.35 (1 H, s), 7.22 (1 H, d, $J_{8,9}$ = 8.8 Hz), 4.34 (7 H, m, H1), 3.3–3.1 (28 H, m, H3, H6, H5), 3.0–2.8 (14 H, m, H2, H4); ^{13}C NMR (D_2O) δ 160.9, 157.6, 150.2, 137.6, 137.0, 136.4, 135.2, 133.1, 130.3, 117.3 (carbons of flavin), 101.4 (C1), 99.2 (C'1), 81.2 (C4), 79.6 (C'2), 73.3 (C3), 72.0 (C5), 71.5 (C2), 60.3 (C6), 33.0 (CH_3); INEPT ^{13}C NMR (D_2O) δ 160.9, 157.6, 150.2, 137.6, 136.4, 135.2, 133.1, 60.3 (negative peaks for C or CH_2), 137.0, 130.3, 117.3, 101.4, 99.2, 81.2, 79.6, 73.3, 72.0, 71.5, 33.0 (positive peaks for CH or CH_3); UV-vis (H_2O) λ_{max} 265 (ϵ 3.47 × 10^4), 342 (7.42 × 10^3), and 435 nm (ϵ 9.38 × 10^3). Anal. Calcd for $C_{48}H_{68}O_{32}N_4\cdot 9H_2O$: C, 41.92; H, 6.30; N, 4.07. Found: C, 41.58; H, 5.89; N, 4.15.

Acknowledgment. We gratefully acknowledge the financial support from the University of Missouri—St. Louis, Mallinckrodt Specialty Chemicals Company, the Missouri Research Assistance Act, and the donors of the Petroleum Research Fund, administered by the American Chemical Society. We thank the National Science Foundation for an instrumentation grant (CHE-8506671).

Supplementary Material Available: ^{1}H NMR spectra of **7–9, 11–13, 15, 17,** and **18**; ^{13}C NMR spectra of **7–9, 12, 15, 17,** and **18**; and ^{13}C INEPT NMR spectra of **8, 9, 12, 15, 17,** and **18** (25 pages). Ordering information is given on any current masthead page.

3-Vinylcoumarins and 3-Vinylchromenes as Dienes. Application to the Synthesis of 3,4-Fused Coumarins and Chromenes

Toru Minami,* Yasuyuki Matsumoto, Seigo Nakamura, Shinichiro Koyanagi, and Masahiko Yamaguchi

Department of Applied Chemistry, Kyushu Institute of Technology, Sensuicho, Tobata, Kitakyushu 804, Japan

Received May 7, 1991

The reaction of α-(diethylphosphono)-γ-butyrolactones **1** with *o*-hydroxyaryl aldehydes **2** and **7** gave 3-(2-hydroxyethyl)coumarins **3** in excellent yields. Treatment of **3** or 3-(2-hydroxyethyl)-2,2-dimethylchromenes **11** derived from **3** with triphenylphosphine dibromide led to the corresponding 3-(2-bromoethyl)coumarins **8** or 3-(2-bromoethyl)chromenes **12** in good yields. The Diels–Alder reaction of the 3-vinylcoumarins **13** or the 3-vinylchromenes **31**, generated in situ from treatment of the bromides **8** or **12** with DBU, with a variety of dienophiles **14–19** and **35** produced regiospecific [2 + 4] cycloadducts, 3,4-fused coumarins **20–28** or 3,4-fused chromenes **32–34** and **36** in good to moderate yields.

Although the development of useful synthetic routes to coumarins[1] and chromenes[2] with 3,4-fused ring systems has been widely studied, it is still an interesting subject since they exhibit a variety of physiological activities. We have

0022-3263/92/1957-0167$03.00/0