CD Exciton Chirality Method: Schiff Base and Cyanine Dye-Type Chromophores for Primary Amino Groups

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Abstract: The CD exciton chirality method is a versatile and unambiguous method for the microscale determination of absolute configurations and conformations of organic molecules in solution. It is particularly powerful when two different chromophores are involved since the coupled CD covers a wide spectral range and becomes a fingerprint for that compound. This paper describes the preparation, properties, and applications of three *p*-amino-substituted Schiff base chromophores to derivatize primary NH_2 groups for the exciton chirality method; the Schiff bases are formed in high yield under mild conditions and yield chromophores which also couple with *O*-acyl chromophores. Moreover, protonation of these Schiff bases yields cyanine-type chromophores exhibiting drastically red-shifted (ca. 100 nm) and 2–3-fold intensified CD couplets. The aldehydes *p*-(dimethylamino)benzaldehyde, julolidinecarbaldehyde, and *p*-(dimethylamino)cinnamaldehyde form Schiff bases with aminocyclohexane to yield Chromophores-I, -II, and -III, respectively. These chromophores have the following λ_{max} (ϵ values) for the neutral and protonated species, respectively: Chrom-I, in MeCN 305 nm (24 300), in MeCN/TFA 395 nm (51 700); Chrom-II, in MeCN 331 nm (21 400), in MeCN/TFA 240 nm (48 300); Chrom-III, in MeOH 361 nm (37 000), in MeOH/TFA 460 nm (64 500). NH_2 groups are converted into Schiff bases without protection of OH groups; in cases where N,O coupling was required, the OH was converted into the (4-methoxyphenyl)-2,4-pentadienoate (Chrom-IV), in MeOH 333 nm (40 000). All neutral Schiff bases and protonated Schiff bases of (1*R*,2*R*)-*trans*-cyclohexanediamine gave exciton-split CD curves with intense amplitudes. However, in the case of protonated Chrom-III, inversion of the CD sign occurred in 45 min at room temperature in methanol and hence the CD should be measured soon after protonation; NMR and MM2 calculations show that the imminium bond in one of the protonated Schiff base chromophore undergoes an $E \rightarrow Z$ isomerization. Derivatives with Chrom-I and -II do not isomerize, and amplitudes of the exciton couplet remain unchanged. Furthermore, the original substrate may be recovered quantitatively when derivatized with Chrom-I. Intense exciton coupling is still observed between chromophores with absorption maxima as far apart as 134 nm.

Introduction

The circular dichroic (CD) exciton chirality method is a versatile microscale technique for determining the absolute configurations and conformations of chiral compounds.[1] Hydroxyl groups are converted into the corresponding esters with *p*-substituted benzoates and other chromophores,[2] upon which interactions between the electric transition moments of chromophores located nearby in space give rise to CD curves exhibiting split Cotton effects. Primary amino groups can also be derivatized with chromophoric groups for CD studies, and chromophores that have been previously described include benzamide,[3] *p*-(dimethylamino)cinnamide,[4] *p*-methoxycinnamide,[5] and 9-anthramide.[6]

In addition to these amides, other chromophores such as 1-aryltriazine[7] and the salicylidenimino Schiff base[8] have been used for CD studies. We have recently developed several red-shifted Schiff base chromophores,[9] a full account of which is described in the following. The advantages of Schiff base chromophores are notable: (i) the amino groups can be selectively derivatized to their corresponding Schiff bases in the presence of free hydroxyl groups; (ii) protonation yields cyanine-type chromophores[5] that give rise to intense and sharp absorption bands at long wavelengths; and (iii) unlike the amide chromophores, the original amines can be recovered if desired through hydrolysis under mild conditions.

The interaction of chromophores with different absorption maxima, i.e., hydroxyl or amino groups acylated with two different chromophores, or the interaction of acylated hydroxyls with neutral or protonated Schiff bases gives rise to fingerprint CD curves spanning the region of the two chromophoric maxima; such fingerprint couplets are diagnostic of the substitution patterns, an extensively studied case being the hexopyranoses.[10]

The recently described Schiff base chromophores, Chrom-I, -II, and -III (Scheme 1, n for neutral and p for protonated species), possess the desirable properties summarized above and yield exciton-split CD curves with signs in accordance with the stereochemistry at the N-bearing stereogenic centers.[9] Primary amines are derivatized with the chromophoric aldehyde reagents to generate the neutral Schiff bases **1n–3n**, which possess strong absorptions at long wavelengths. Treatment of these derivatives with trifluoroacetic acid (TFA) creates new species, the protonated Schiff bases **1p–3p**, with 2–3-fold increases in the molar extinction

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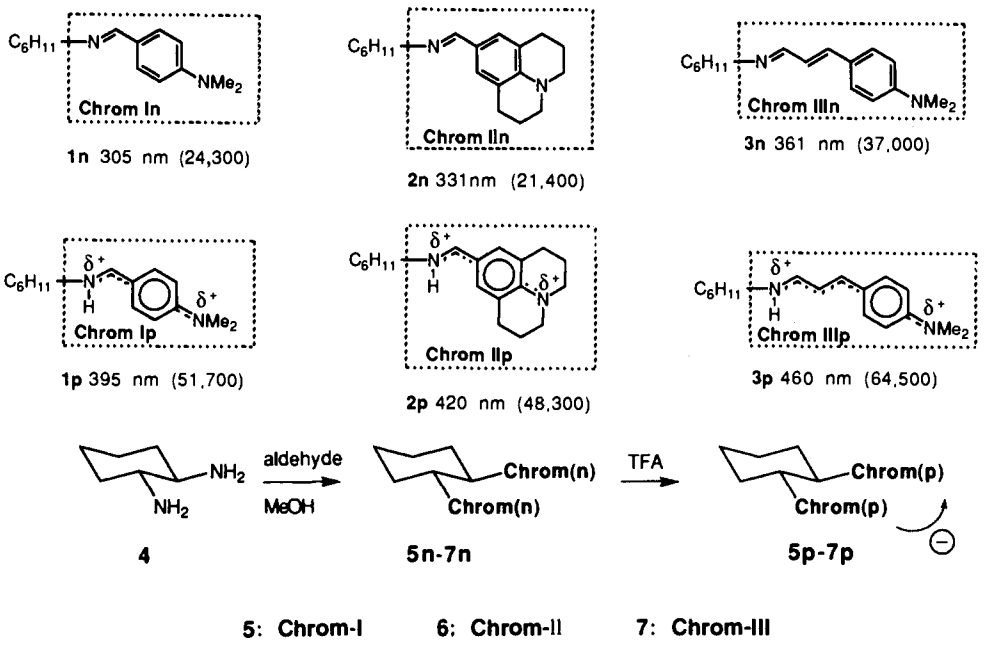
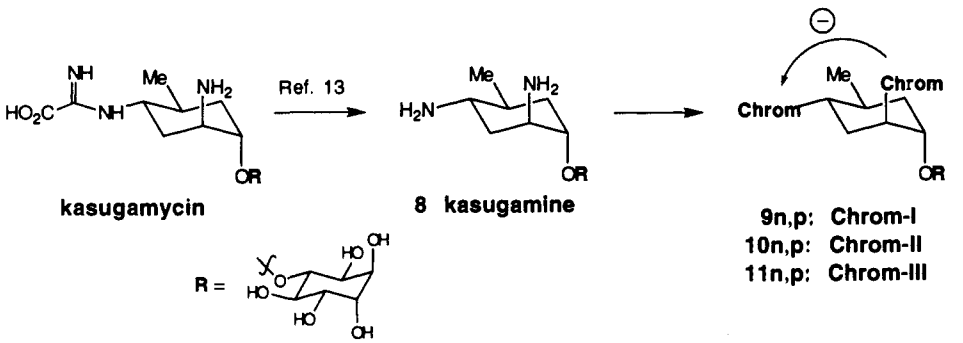
Scheme 1



Scheme 2



coefficients ϵ over those of the neutral species (hyperchromic effect) and bathochromic shifts of up to 100 nm in λ_{max}. By virtue of their unusually intense and red-shifted absorbances, these chromophores show promise for use with natural products containing chromophoric groups that could otherwise overlap and interfere with the CD analysis. Strong absorbance results in strong split Cotton effects (amplitude A is approximately proportional to the square of ϵ),[12] and this greatly enhances the sensitivity and ease of measurements.

Despite these advantages, there are several conformational and configurational issues associated with these chromophores that need to be addressed for accurate interpretation of the CD data. The unexpected CD properties exhibited by certain cyanine dye chromophores[5] and the retinilydenimine and hexadienylidenimine derivatives of chiral 1,2-cyclohexanediamine have been reported.[11] These conjugated alkyl azomethine chromophores were found to be unsuited for determining the correct stereochemistry at the chiral centers, since in their preferred conformations, their electric transition moments are not parallel to the C–N bonds; this led to exciton-split CD curves having signs opposite to that expected from the original configuration of the amino groups.

It was also observed that formation of the protonated Schiff base derivative (compound **7p**) with Chrom-III gave a CD curve for which the sign reversed itself over time. Namely, the initially observed CD curve is the one expected on the basis of the orientation of the C–N bond in the substrate; however, there is a gradual isomerization of the protonated imino group of one of the chromophores that manifests itself in sign reversal of the CD exciton couplet. Such configurational changes, however, were not observed with Chrom-I and -II.

In this report, we present a full account of the studies on the chemistry and CD properties involving Chrom-I, -II, and -III and provide evidence and rationalization of the unexpected isomerization phenomenon of Chrom-III.

Synthesis of Schiff Base Chromophores

The neutral Schiff base derivatives were prepared by reacting various amines with the three chromophoric aldehydes, *p*-(dimethylamino)benzaldehyde (*p*-DMBA, Chrom-I), julolidine aldehyde (Chrom-II), and *p*-(dimethylamino)cinnamaldehyde (*p*-DMCA, Chrom-III).[9] The derivatization of aminocyclohexane and the chiral amine (1*R*,2*R*)-*trans*-cyclohexanediamine **4** is shown in Scheme 1. The bis derivatives **5n–7n** were prepared in one step. The 1,3-diamine **8** derived from kasugamycin[13] was converted directly into the bis-Schiff bases **9–11** without protection of the hydroxyl groups (Scheme 2), while the amino sugars methyl α-L-acosaminide and methyl β-L-daunosaminide were transformed to derivatives **13** and **15**, respectively (Scheme 3). In these examples, Chrom-III was used to derivatize the amino groups and (*p*-methoxyphenyl)pentadienoate[9] (Chrom-IV) for the hydroxyl groups. The above reactions were performed readily in high yields by stirring in MeOH with anhydrous Na_2SO_4 at room temperature or under reflux. The corresponding protonated Schiff bases were generated by addition of an excess of trifluoroacetic acid to an acetonitrile solution of the neutral species directly into the UV or CD cell.

The progress of the Schiff base formation between diamine **4** and *p*-DMBA (λ_{max} 335 nm, MeCN) or *p*-DMCA (λ_{max} 387 nm,

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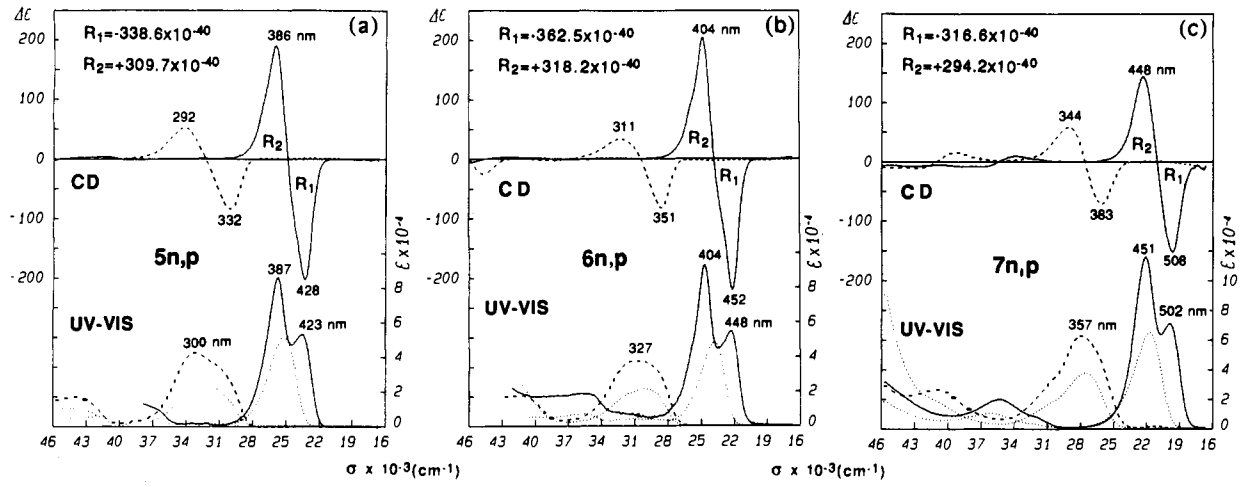
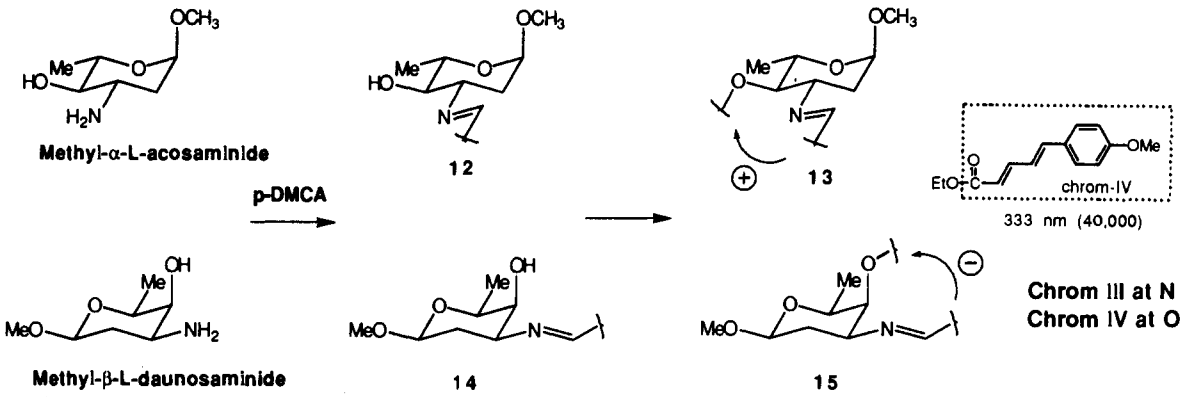


Figure 1. UV–vis, CD spectra, and rotational strengths R (cgs units) in acetonitrile (a,b) and in methanol (c) of the bis-Schiff bases **5–7** with Chrom-I, -II, and -III: neutral species (n) shown by dashed lines and protonated species (p) by solid lines. The UV–vis spectra of the mono-Schiff base derivatives **1n,p-3n,p** are shown in a–c in dotted lines.

Scheme 3



MeOH) to afford **5n** and **7n**, respectively, was followed by measurement of the unreacted aldehyde species. Namely, addition of TFA to the reaction mixture yielded the bathochromically shifted species **5p** and **7p**, thus removing the absorption overlaps between the neutral Schiff base and the aldehyde. The reaction of diamine **4**, 10^{-2} M diamine concentration with Chrom-I, required 10 h at room temperature, with a $t_{1/2}$ of 1.5 h. The reaction of **4** with Chrom-III proceeded rapidly under the same conditions and was complete after 3 h, with a $t_{1/2}$ of 15 min. The feasibility of recovering the starting material was studied with bis derivative 5; hydrolysis of the diimine under mildly acidic conditions showed quantitative recovery of the starting diamine.

UV–Vis and CD Spectra of the Mono- and Bischromophoric Derivatives

The UV–vis spectra of the monoderivatives **1n–3n** (Chrom-I, -II, and -III) are characterized by symmetric intense bands attributed to a strong π,π^* intramolecular charge-transfer transition exhibiting λ_{max} at 305 nm (ϵ 24,300), 331 nm (ϵ 21 400), and 361 nm (ϵ 37 500), respectively. Addition of TFA to the solutions of **1n–3n** yields the protonated Schiff bases **1p–3p**, resulting in drastic bathochromic shifts of up to 100 nm for all chromophores and 2–3-fold increases in ϵ values by formation of cyanine-type dyes (Scheme 1). UV–vis spectra of bis Schiff bases **5n–7n** display broad, intense bands with λ_{max} values in the 300–360-nm range (Figure 1); both bathochromic and hyperchromic shifts were observed upon addition of TFA, leading to the bis-protonated species **5p–7p**. As previously observed with the cyanine dye chromophore,[5] the UV–vis spectra of these protonated derivatives are characterized by two distinct absorbance bands that arise from the exciton coupling between the two chromophores of the derivatives: the energy gap between the α and β excited states is large enough to give rise to two split bands in the long-wavelength region. For **5p–7p**, the separations between the bands are 36, 44, and 51 nm, respectively (Figure 1). In exciton-split cases, the intensity ratio of the two absorption bands depends on the dihedral angle θ between the transition moments of the chromophores;[14] when θ is greater than 90°, the first band at longer wavelengths is stronger than the second band, while this is reversed when the angle is less than 90°. From the intensity ratio of the two bands (Figure 1), θ is shown to be less than 90° for **5p–7p**. The separation of the absorbance bands is not as distinct in the case of the 1,3-diamine derivatives **9p–11p** (Table 1 and Figure 2) because the larger distance between the two 1,3-chromophores leads to a weaker coupling. In these examples, the first band at longer wavelengths is stronger than the second, and thus θ is greater than 90°.

All neutral 1,2-diamine derivatives **5n–7n** displayed strong exciton-split CD curves (Figure 1) with large negative A values (Table 1). The negative exciton couplets with negative first, at longer wavelengths, and positive second Cotton effects are in agreement with the negative chirality or dihedral angle between the two C–N bonds of the starting (1*R*,2*R*)-*trans*-cyclohexanediamine. 1,3-Diamine derivatives **9–11** also displayed CD curves with the expected negative signs (Table 1 and Figure 2); although the A values are still intense, they are smaller than those of **5–7** because of the longer 1,3-interchromophoric distances. In both sets of examples, derivatives **7** and **11** functionalized with Chrom-III show the smallest exciton splitting even though the ϵ value for this chromophore is larger than those for Chrom-I and -II. This undoubtedly results from the presence of the extra double bond in Chrom-III which increases the length of the

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Table 1. UV–Vis and CD Data of Neutral (n) and Protonated (p) Bischromophoric Derivatives **5**–**7** and **9**–**11** and the Bischromophoric Amino Sugar Derivatives **13** and **15**[a]

compd	UV λ_{max} (ϵ)	CD λ_{ext} ($\Delta\epsilon$)	A
5n[b]	300 (43 000)	332 (–83.1)/292 (+53.4)	–136
5p[b]	423 (53 300)/387 (86 100)	428 (–202.2)/386 (+190.4)	–392
6n[b]	327 (37 500)	351 (–83.0)/311 (+33.6)	–117
6p[b]	448 (54 800)/404 (93 400)	452 (–220)/404 (+204.0)	–424
7n[c]	357 (63 000)	383 (–70.2)/344 (+58.3)	–128
7p[c]	502 (71 000)/451 (116 000)	508 (–151.0)/448 (+144.0)	–295
9n[c]	325 (48 000)	334 (–56.2)/294 (+26.6)	–83
9p[c]	411 (93 800)	417 (–151)/383 (+110.0)	–261
10n[c]	344 (45 000)	354 (–51.3)/314 (+17.8)	–69
10p[c]	435 (93 400)	440 (–153)/404 (+112)	–265
11n[c]	368 (63 000)	385 (–23.1)/348 (+15.5)	–39
11p[c]	484 (93 000)	498 (–94.4)/450 (+84.4)	–178
13p[c]	342 (40 000)/476 (64 500)	469 (+35.0)/350 (–25.0)	+60
15n[c]	340 (61 000)	369 (–44.8)/327.5 (+24.3)	–69
15p[c]	339 (43 200)/474 (64 000)	471 (–25.3)/352 (+12.2)	–38

[a] The A values (amplitudes) are the differences in $\Delta\epsilon$ values of the two extrema of split CD curves. The sign of A corresponds to the sign of the Cotton effect at longer wavelengths. [b] MeCN. [c] MeOH.

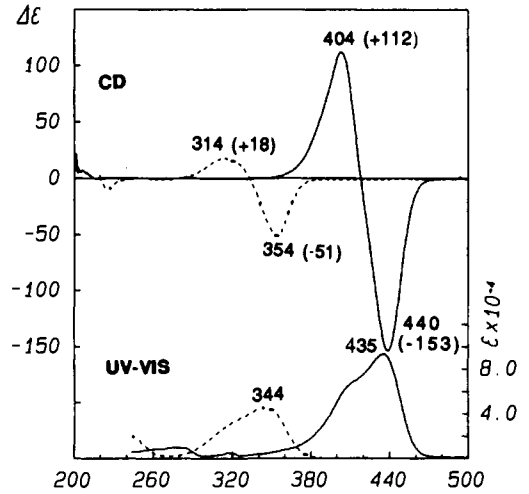

Figure 2. UV-vis and CD spectra of the neutral (**10n**, dashed line) and protonated (**10p**, solid line) Chrom-II derivatives of kasugamine in acetonitrile. The $\lambda_{max}(\Delta\epsilon)$ and $\lambda_{max}(\epsilon)$ values are also shown.

chromophore and hence the interchromophoric distance; thus, it should be noted that longer chromophores with stronger absorptions do not necessarily give larger A values.

The CD spectra of the protonated forms **5p**–**7p** are compared with the neutral forms in Figure 1. As with the UV–vis absorbance bands, the CD bands undergo hyperchromic and bathochromic shifts upon protonation. The 3–4-fold enhancement in the A values correlates well with the expected values arising from doubling the intensity of the UV–vis absorbance bands. Similar shifts were also noted in the cases of 1,3-protonated diamine derivatives **9**–**11**. In these examples, the λ_{ext} of the two CE's matches well the λ_{max} of the two bands in the corresponding UV–vis spectra; this confirms the exciton coupling origin of the UV–vis band splitting.

In all three cases **5**–**7**, the bathochromic shift observed upon protonation results in almost conservative couplets, evidenced by the similar absolute values of the rotational strengths R_1 and R_2 (Figure 1). This is different from the neutral forms where partial overlap with other bands decreases the R value of the second Cotton effect. In both the neutral and protonated derivatives of **5**–**7**, the negative signs of the CD curves correctly represent the absolute sense of twist between the C–N bonds in model diamine **4**. In the cases of Chrom-I (**5**, **9**) and Chrom-II (**6**, **10**), the negative couplets of both neutral and protonated forms do not show any change in the intensity over the time. This conformational stability of Chrom-I and -II is also supported by NMR and MM2 calculations.

Isomerization of Chrom-III

An unexpected change in the CD spectra was observed with compound **7p** (Chrom-III). After addition of TFA to the solution of **7n** in methanol,[15] a strong negative exciton couplet was obtained. However, the intensity of the bands decreased over time and eventually changed signs so that 45 min after addition of TFA, a positive CD couplet remained [506 nm ($\Delta\epsilon$ +53.5), 449 nm (–51.6), A +105.1] (Figure 3). The UV–vis bands, on the other hand, showed only minor changes in the relative intensity of the two bands [502 nm (ϵ 78 500)/450 nm (ϵ 108 000)]. While intensity changes could arise from slight conformational alterations, the sign reversal of the CD couplet indicates an inversion of the absolute twist between the two chromophores in **7p**, resulting from some major conformational and/or configurational changes in the molecule. ^{1}H NMR studies as well as molecular mechanics calculations were performed in order to clarify this aspect.

^{1}H NMR spectra for compounds **5n**–**7n** show only one set of signals for the chromophores and the methine hydrogens H_1 and H_2, indicating that the molecules are C_2-symmetric (Figure 3, **7n**). The large coupling constant for the olefinic hydrogens (H_b and H_c) of **7n** indicates a *trans* double bond, while the absence of NOE between H_a and H_b hydrogens indicates an *s-trans* conformation between the imino and olefinic double bonds. In **6n** and **7n**, the NOESY peaks observed for the imino hydrogen H_a and the methine hydrogen(s) H_1 (and H_2) showed that the two hydrogens were *syn* oriented, i.e., the neutral imino group adopts an E configuration. These data are consistent with the expected low-energy conformation with the chromophores fully extended with E configurations for both the C=N and C=C bonds (for Chrom-III). Molecular mechanics calculations also indicated the preference for the E/E configurations of the two C=N bonds over the E/Z and Z/Z configurations.[16] With the above orientations, the long axis of each of the chromophores is aligned along the C–N bond, and the absolute chirality between the interacting chromophoric groups giving a negative CD couplet agrees with that of the original amino groups.

Upon protonation, ^{1}H NMR data indicate that the C_2 symmetry of the bis-protonated Schiff base derivatives **5p** and **6p** does not change over time. With compound **7**, however, the ^{1}H NMR spectrum in methanol-d_4 shows the presence of a sole C_2-symmetric species **7p** immediately following TFA addition. However, a new set of signals develop over time corresponding to the non-C_2-symmetric species **7p**$_1$ (Figure 3). After 45 min, an equilibrium ratio of 45:55 is obtained for **7p** and **7p**$_1$, as calculated from the ^{1}H NMR spectrum according to the expression

$$\%\mathbf{7p} = [A(H_a)/[A(H_a) + (H_{a'}) + A(H_{a''})]] \times 100$$

where $A(H_a)$, $A(H_{a'})$, and $A(H_{a''})$ are the areas of two H_a and one each $H_{a'}$ and $H_{a''}$ hydrogens, respectively. The structure of **7p**$_1$ is assigned on the basis of NOE data showing that one of the C=N bonds has isomerized to the Z configuration: the NOESY plot shows cross relaxation peaks for species **7p**$_1$ between $H_2/H_{a''}$ (*trans* chromophore) and between $H_1/H_{b'}$ (*cis* chromophore) (Figure 4). The latter cross peak arises from the close proximity of these two hydrogens brought together by the Z C=N bond and is not seen for the E chromophore.

Species **7p**$_1$ possesses a positive chirality between the two chromophores and gives rise to a strong positive CD curve that leads to the sign reversal in the CD curve for compound **7**. Addition of a base such as triethylamine to the solution of **7p** and **7p**$_1$ immediately regenerates the original C_2-symmetric species **7n**, as evident from the observed CD curve (Figure 3). ^{1}H NMR also showed regeneration of the C_2-symmetric **7n** upon addition

(15) The change in the CD was observed in other solvents as well: the times required for the spectra to change and stabilize to a new one in acetonitrile, DMSO, and dichloromethane were 6 min, 2 h, and 6 h, respectively.

(16) Minimum energy conformations were obtained by simulated annealing stochastic dynamics going from 300 to 0 K over 10 ps with a time constant of 1 fs using the amber force field.

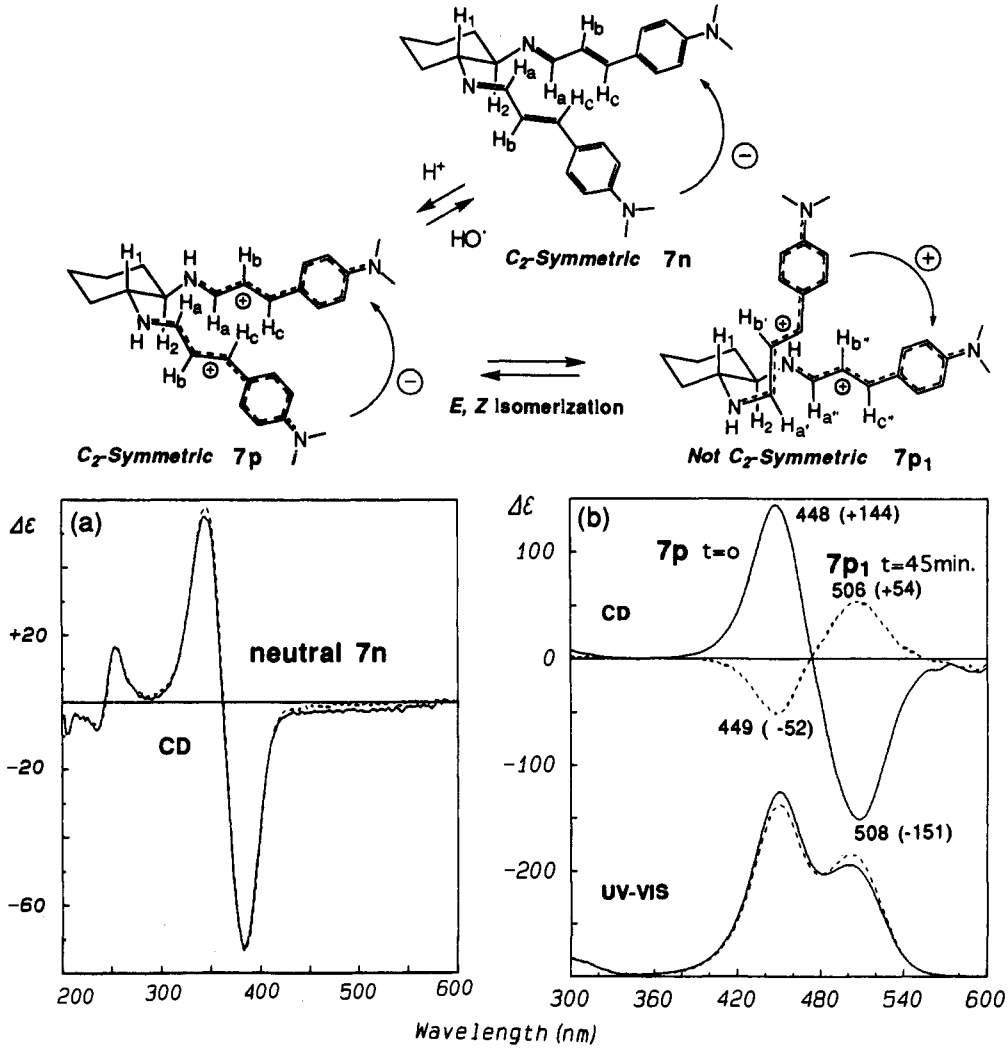

Figure 3. Top: *E,Z* Isomerization of C=N bond in bis-derivative **7n** (Chrom-III) in methanol after addition of TFA. Bottom: (a) CD spectra of the original **7n** (solid line) and after neutralization of **7p** + **7p**$_1$ with base (dashed line); (b) CD and UV–vis spectra of **7p** immediately after addition of TEA to **7n** (solid line) and after 45 min (dashed line).

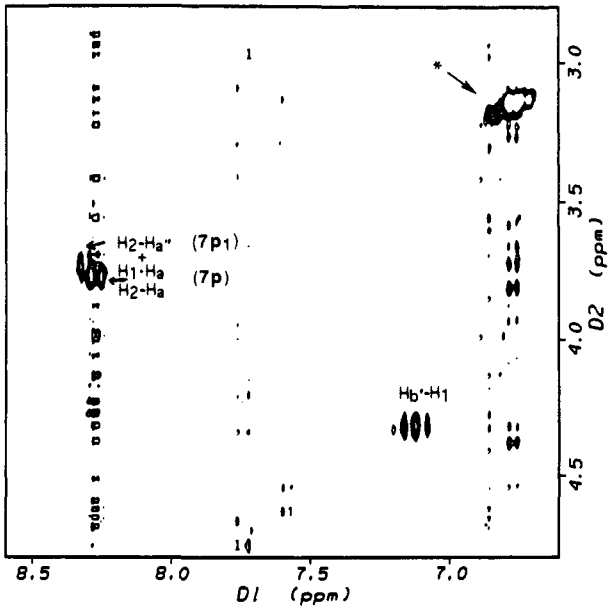

Figure 4. Expanded region of the NOESY spectrum in CD_3OD of the mixture **7p** + **7p**$_1$ (see Figure 3). The diagnostic cross peaks between the methine hydrogens and the olefinic hydrogens are shown. * represents the cross peak between N-Me and aromatic hydrogens.

of the base. The regenerated neutral form **7n** follows the same isomerization kinetics upon readdition of TFA. Isomerization of one of the imine bonds to the *Z* configuration presumably results from the electrostatic repulsion between the two positively charged side chains and is facilitated by the decrease in double bond character of the imino group upon its protonation. With Chrom-I and -II, however, isomerization of the imino groups is not observed, undoubtedly because of the steric hindrance, if isomerization were to occur, arising from the juxtaposition of the ortho-aromatic hydrogens and the methine hydrogens H_1 or H_2 of the cyclohexane ring.

Applications to Amino Sugars

One advantage of using Schiff base derivatives is that amino groups can be selectively functionalized in the presence of free hydroxyl groups. Derivatization of 1,3-diamine in kasugamine (**8**) was achieved without interference from the hydroxyl group of the inositol moiety. Therefore, natural products such as amino sugars are readily studied using Schiff base chromophores. In our previous report,[9] we described the preparation of methyl α-L-acosaminide derivative **13**, possessing both a Schiff base chromophore and an acyl chromophore. Thus, the use of two chromophores, which selectively derivatize amino and hydroxyl groups, allows one to study the exciton interaction between neutral and protonated Schiff base chromophores and other types of chromophores, such as acylates. A further example of coupling between O,N-derivatives is given here. Methyl β-L-daunosaminide was derivatized with Chrom-III at the amino group and then Chrom-IV at the hydroxyl group to give the derivative **15** (Scheme 3). In the neutral Schiff base form, the two

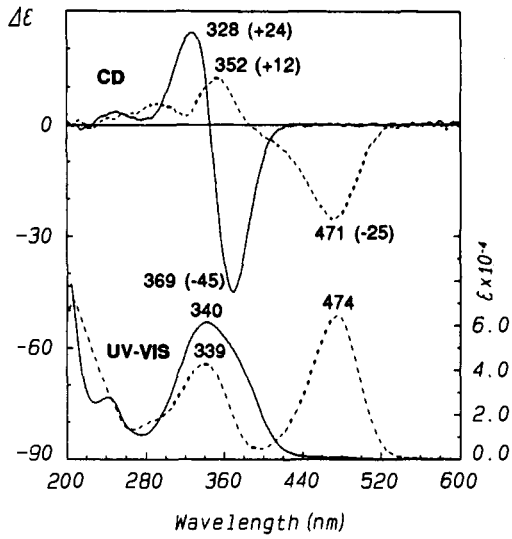

Figure 5. UV-vis and CD spectra of the methyl β-L-daunosaminide derivatives (Chrom-III and Chrom-IV) in methanol: neutral **15n** (solid line) and protonated **15p** (dashed line). The $\lambda_{max}(\Delta\epsilon)$ and $\lambda_{max}(\epsilon)$ values are also shown.

chromophores have similar absorbance maxima, and the CD spectrum of **15** therefore resembles those resulting from coupling of two identical chromophores [**15**: 369 nm (–45) and 328 nm (+24), *A* –69, Figure 5].

Protonation of Chrom-III with TFA generates a new bathochromically shifted band at 474 nm along with the 339-nm band from Chrom IV (Figure 5). The separation of the two bands in the UV–vis spectrum enables one to calculate the concentration of compounds on a microscale level from the known extinction coefficients of the electronic spectra. Although the difference in λ_{max} between the two chromophores is 134 nm (340 and 474 nm), an exciton coupling with amplitude of 37 (–25 at 471 nm and +12 at 352 nm, Figure 5) is still observed. This is so far the largest separation in λ_{max} for which a significant *A* value is observed.

Conclusions

Chromophores I-III have been shown to be very useful for stereochemical assignments of compounds containing primary amino groups. All three chromophores in their neutral form display intense exciton-split CD curves, the signs of which correctly represent the absolute sense of twist between the amino groups. Moreover, protonation results in strong bathochromic and hyperchromic shifts that further enhance the sensitivity and the spectral range for study. Although the derivatives with protonated Chrom-III display the most red-shifted bisignate Cotton effects, isomerization of the C=N bond that occurs with time makes this chromophore unsuited for CD studies if measurements cannot be performed immediately after protonation. In contrast, derivatives with protonated Chrom-I and -II do not isomerize, and intensities as well as the signs of exciton couplets remain unchanged. The original amines may be recovered by hydrolysis of Chrom-I. Finally, because of the strong absorbance of these chromophores, CD exciton coupling can still be observed even when the absorbance maxima of the chromophores are separated by as much as 135 nm.

Experimental Section

The UV spectra were recorded on a Perkin Elmer Lambda 4B UV–spectrophotometer and the CD spectra on a Jasco 720 spectropolarimeter; smoothing and other manipulations were carried out using software developed in-house (DFT = discrete Fourier transform, procedure for smoothing). The optical rotations data were obtained using a Jasco DIP-181 digital polarimeter. ^{1}H-NMR spectra were recorded with a Varian VXR 400-MHz instrument. Steady-state NOE was measured on VXR-300 and -400 at room temperature. To get a steady-state condition, the irradiation time was set to 30 s. NOESY experiments were carried out on Varian VXR-400 and Brucker AM-300 instruments at room temperature. Mixing times were 1 s (400 MHz) or 250 ms (300 MHz), and relaxation delays were set to 3 s (400 MHz) and 2 s (300 MHz). Data matrix was $1k \times 1k$ in absolute intensity mode at 400 MHz and $2k \times 256$ (real and imaginary each) in State's phase sensitive mode at 300 MHz. Trifluoroacetic acid (TFA), trifluoroacetic acid-*d* (for protonation prior NMR measurements), *p*-(dimethylamino)cinnamaldehyde (DMCA), aminocyclohexane, and (*R*,*R*)-1,2-diaminocyclohexane were purchased from Aldrich and used without further purification.

***N*-[[4-(Dimethylamino)phenyl]methylene]cyclohexanamine (1).** A solution of cyclohexylamine (50 μL, 0.44 mmol) and *p*-(dimethylamino)-benzaldehyde (65 mg, 0.44 mmol) in anhydrous methanol (2 mL) was stirred at room temperature in the presence of anhydrous Na_2SO_4. After 4 h, the mixture was filtered through glass wool and was concentrated to afford a pale yellow solid (101 mg, quantitative) which was essentially pure imine. The residue was recrystallized from hexane–ethyl acetate in the presence of triethylamine: ^{1}H-NMR ($CDCl_3$) δ 1.16–1.41 (m, 4H, CH_2), 1.53–1.84 (m, 6H, CH_2), 3.01 (s, 6H, NCH_3), 3.10 (m, 1H, CHN), 6.72 (d, J = 8.8 Hz, 2H, aromatic), 7.61 (d, 2H, aromatic), 8.19 (s, 1H, olefinic); FAB-MS (*m*-nitrobenzyl alcohol) m/z 231 $(M + 1)^+$; HRMS for $C_{15}H_{22}N_2$ calcd 230.1783, found 230.1774. After protonation: ^{1}H-NMR ($CDCl_3$) δ 1.23 (m, 1H, CH_2), 1.36 (q, 2H, CH_2), 1.61 (q, 2H, CH_2), 1.72 (d, 1H, CH_2), 1.92 (d, 2H, CH_2), 2.08 (d, 2H, CH_2), 3.20 (s, 6H, NCH_3), 3.58 (br, 1H, CHN), 6.76 (d, J = 8.7 Hz, 2H, aromatic), 7.76 (d, J = 8.7 Hz, 2H, aromatic), 7.84 (d, J = 15.4 Hz, 1H, olefinic), 10.56 (br s, 1H, N*H*).

***N*-[(2,3,6,7-Tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizin-9-yl)methylene]cyclohexanamine (2).** A solution of cyclohexylamine (34 μL, 0.30 mmol) and julolidine aldehyde (60 mg, 0.30 mmol) in anhydrous methanol (3.0 mL) was stirred at room temperature in the presence of anhydrous Na_2SO_4. After 6 h, the mixture was filtered through a pad of celite and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and a white powder product (72 mg, 85%) precipitated by addition of hexane: ^{1}H-NMR ($CDCl_3$) δ 1.15–1.38 (m, 3H, CH_2), 1.48–1.55 (m, 2H, CH_2), 1.60–1.80 (m, 5H, CH_2), 1.90 (m, 4H, CH_2), 2.73 (m, 4H, CH_2Ar), 3.05 (m, 1H, CHN), 3.16 (m, 4H, CH_2N), 7.12 (s, 2H, aromatic), 8.04 (s, 1H, olefinic); FAB-MS (*m*-nitrobenzyl alcohol) m/z 283 $(M + 1)^+$; HRMS for $C_{19}H_{26}N_2$ calcd 282.2096, found 282.2094. After protonation: ^{1}H-NMR ($CDCl_3$) δ 1.34 (q, 2H, CH_2), 1.68 (q, 2H, CH_2), 1.87 (d, 1H, CH_2), 2.02 (d, 2H, CH_2), 2.72 (m, 4H, CH_2Ar), 3.40 (m, 4H, CH_2N), 3.49 (m, 1H, CHN), 7.26 (s, 2H, aromatic), 7.61 (s, 1H, olefinic).

[[3-[4-(Dimethylamino)phenyl]-2-propenylidene]amino]cyclohexane (3). A solution of aminocyclohexane (50 mg, 0.504 mmol) and *p*-(dimethylamino)cinnamaldehyde (88.3 mg, 0.504 mmol) in dry MeOH (6 mL) was stirred at room temperature under argon in the presence of a small amount of Na_2SO_4. After 4 h, the mixture was filtered and the solvent was evaporated. The residue was triturated with ethyl ether to afford **3** as a yellow solid (121 mg, 94%): ^{1}H-NMR (DMSO-d_6) δ 1.1–1.44 (m, 5H), 1.53–1.65 (m, 3H), 1.66–1.78 (m, 2H), 2.95 (s, 6H, N-CH_3), 3.0 (m, 1H), 6.62 (dd, $J_{H_b-H_a}$ = 8.9 Hz, $J_{H_b-H_c}$ = 16 Hz, H_b), 6.7 (d, 2H, J = 8.8 Hz, H_m), 6.9 (d, J = 16 Hz, H_c), 7.4 (d, 2H, J = 8.8 Hz, H_o), 8.0 (d, J = 8.9 Hz, H_a); CI-MS 257 $(M + 1)^+$; ^{1}H-NMR (CD_3OD) δ 1.15–1.55 (m, 5H), 1.66–1.77 (m, 3H), 1.78–1.88 (m, 2H), 3.10 (m, H_1), 6.65 (dd, $J_{H_b-H_a}$ = 8.95 Hz, $J_{H_b-H_c}$ = 16.0 Hz, H_b), 6.72 (d, J = 8.9 Hz, 2H, H_m), 7.00 (d, J = 15.8 Hz, H_c), 7.04 (d, J = 8.87 Hz, 2H, H_o), 8.03 (d, J = 9.03 Hz, H_a). After protonation: ^{1}H-NMR (DMSO-d_6) δ 1.10–1.50 (m, 5H), 1.54–1.64 (m, 1H), 1.72–1.82 (m, 2H), 1.90–2.10 (m, 2H), 3.10 (s, 6H, N-CH3), 3.65 (m, H_1), 6.8 (d, J = 8.96 Hz, H_m), 6.83 (dd, $J_{H_b-H_a}$ = 10.5 Hz, $J_{H_b-H_c}$ = 14.8 Hz, H_b), 7.6 (d, J = 9.0 Hz, H_o), 7.74 (d, J = 14.8 Hz, H_c), 8.48 (dd, $J_{H_a-H_b}$ = 10.5 Hz, J_{H_a-NH} = 15.0 Hz); ^{1}H-NMR (CD_3OD) δ 1.18–1.60 (m, 5H), 1.65–1.78 (m, 1H), 1.81–1.93 (m, 2H), 1.95–2.10 (m, 2H), 3.12 (s, 6H, N-CH_3), 3.62 (m, H_1), 6.81 (d, J = 9.0 Hz), 6.81 (dd, $J_{H_b-H_a}$ = 10.7 Hz, $J_{H_b-H_c}$ = 14.7 Hz, H_b), 7.61 (d, J = 9.0 Hz, 2H, H_o), 7.74 (d, J = 14.8 Hz, H_c), 8.36 (d, J = 10.5 Hz, H_a).

(*R*,*R*)-*N*,*N*'-Bis[[4-(dimethylamino)phenyl]methylene]-1,2-cyclohexanediamine (5). A solution of (*R*,*R*)-1,2-diaminocyclohexane (4.2 mg, 0.037 mmol) and *p*-(dimethylamino)benzaldehyde (11.0 mg, 0.074 mmol) in anhydrous methanol (0.5 mL) was allowed to stir for 12 h at room temperature in the presence of anhydrous Na_2SO_4. After filtration and concentration, the residue was dissolved in ethyl acetate with a few drops of triethylamine, and hexane was added to precipitate the product as a white solid (12.6 mg, 91%). The product can be recrystallized from ethyl acetate and hexane in the presence of triethylamine to afford white needles: ^{1}H-NMR ($CDCl_3$) δ 1.47 (t, 2H, CH_2), 1.83 (m, 6H, CH_2), 2.96 (s, 12H, NCH_3), 3.32 (m, 2H, CHN), 6.60 (d, J = 8.8 Hz, 4H,

aromatic), 7.47 (d, J = 8.7 Hz, 4H, aromatic), 8.09 (s, 2H, olefinic); FAB-MS (*m*-nitrobenzyl alcohol) m/z 377 $(M + 1)^+$; HRMS for $C_{24}H_{32}N_4$ calcd 376.2627, found 376.2643; mp = 148.5–149.5 °C (uncorrected). After protonation: ^{1}H-NMR ($CDCl_3$) δ 1.45 (t, 2H, CH_2), 1.80 (m, 2H, CH_2), 1.93 (d, 2H, CH_2), 2.18 (d, 2H, CH_2), 3.17 (s, 12H, NCH_3), 4.04 (br, 2H, CHN), 6.68 (d, J = 8.6 Hz, 4H, aromatic), 6.72 (d, 4H, aromatic), 8.22 (br, 2H, olefinic), 10.67 (br, 2H, NH).

Hydrolysis of 5. To the solution of the bis-Schiff base (67 mg, 0.18 mmol) in acetonitrile (9 mL) was added trifluoroacetic acid (90 μL, 0.54 mmol) then water (9 mL). After 1 day of stirring at room temperature, the solution was concentrated, diluted with water (ca. 20 mL), and washed with dichloromethane (ca. 3 × 10 mL). The aqueous layer was evaporated and pumped dry to yield the bis-TFA salt of (*R,R*)-1,2-diaminocyclohexane as a pale yellow solid (61 mg, quantitative): ^{1}H-NMR (D_2O) δ 1.15 (br-quint, 2H, γ-CH), 1.32 (br-quart, 2H, β-CH), 1.61 (br-d, 2H, γ-CH), 1.95 (br-d, 2H, β-CH), 3.15 (br-d, 2H, α-CH); mp = 193 °C (decomp); $[\alpha]^{24}_{435}$ = –15.4 (*c*, 0.26 H_2O) [an authentic diamine **4**, bis-TFA salt $[\alpha]^{24}_{435}$ = –15.8 (*c*, 0.26 H_2O)].

Rate of Reaction of (*R,R*)-1,2-Diaminocyclohexane with *p*-(Dimethylamino)benzaldehyde and *p*-(Dimethylamino)cinnamaldehyde at 10^{-2} M and 10^{-3} M. (a) A solution of (*R,R*)-1,2-diaminocyclohexane (1 × 10^{-2} M, 10 μmol) and *p*-(dimethylamino)benzaldehyde (2 × 10^{-2} M, 20 μmol) in 1 mL of methanol was stirred at room temperature over anhydrous Na_2SO_4. Aliquots (10 μL, 0.1 μmol) were taken, diluted with a TFA solution (1.5 × 10^{-4} M, 10 mL, 1.5 μmol) and analyzed by UV. The analysis indicated that the reaction was complete after ca. 10 h. The time for 50% conversion ($t_{1/2}$) was ca. 1.5 h. (b) The reaction was repeated as in a using *p*-(dimethylamino)cinnamaldehyde instead of *p*-(dimethylamino)benzaldehyde. The reaction was complete in *ca.* 3 h with a $t_{1/2}$ of ca. 15 min. (c) A solution of the diamine (1 × 10^{-3} M, 1 μmol) and *p*-(dimethylamino)benzaldehyde (2 × 10^{-3} M, 2 μmol) in 1 mL of methanol was stirred at room temperature over anhydrous Na_2SO_4. Aliquots (5 μL, 0.005 μmol) were taken, diluted with a TFA solution (1.5 × 10^{-4} M, 0.5 mL, 0.075 μmol) in a UV cell, and then analyzed. The reaction required more than 48 h at room temperature with the $t_{1/2}$ *ca.* 4 h. (d) The reaction was repeated as in c using *p*-(dimethylamino)cinnamaldehyde instead of *p*-(dimethylamino)benzaldehyde. The reaction was complete after *ca.* 24 h with the $t_{1/2}$ *ca.* 1 h.

(*R,R*)-*N,N'*-Bis[(2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizin-9-yl)-methylene]-1,2-cyclohexanediamine (6). A solution of (*R,R*)-1,2-diaminocyclohexane (43.5 mg, 0.38 mmol) and julolidine aldehyde (160 mg, 0.79 mmol) in anhydrous methanol (2 mL) was allowed to stir at room temperature for 6 h in the presence of anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, the residue was triturated with ether to afford the product (168 mg, 92%) as a light yellow powder: ^{1}H-NMR ($CDCl_3$) δ 1.65–1.82 (m, 8H, CH_2), 1.86 (m, 8H, CH_2), 2.67 (m, 8H, CH_2Ar), 3.12 (m, 8H, CH_2N), 3.26 (m, 2H, CHN), 6.70 (s, 4H, aromatic), 7.97 (s, 2H, olefinic); FAB-MS (*m*-nitrobenzyl alcohol) m/z 481 $(M + 1)^+$; HRMS for $C_{32}H_{40}N_4$ calcd 480.3252, found 480.3221. After protonation: ^{1}H-NMR ($CDCl_3$) δ 1.40 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 1.96 (m, 8H, CH_2), 2.08 (d, 4H, CH_2), 2.66 (m, 8H, CH_2Ar), 3.40 (m, 8H, CH_2N), 3.85 (m, 2H, CHN), 7.19 (s, 4H, aromatic), 7.85 (s, 2H, olefinic), 11.58 (br-s, 2H, NH). When the olefinic hydrogen (δ 7.85 ppm) was irradiated, the intensity of the CHN hydrogen (δ 3.85 ppm) was increased by 3%.

(*R,R*)-1,2-Bis[[3-[4-(dimethylamino)phenyl]-2-propenylidene]amino]cyclohexane (7). A solution of (*R,R*)-1,2-diaminocyclohexane (68 mg, 0.595 mmol) and *p*-(dimethylamino)cinnamaldehyde (210 mg, 1.19 mmol) in anhydrous MeOH (5 mL) was stirred for 2 h at room temperature in the presence of anhydrous Na_2SO_4. The solution was partially concentrated under reduced pressure, and ether was added to precipitate **7** as a pure yellow solid (yield > 90%): ^{1}H-NMR (CD_3OD) δ 1.41–1.54 (m, 2H), 1.55–1.71 (m, 2H), 1.75–1.9 (m, 4H), 2.95 (s, 12H, N-CH_3), 3.15 (m, 2H, H_1, H_2), 6.57 (dd, $J_{H_b-H_a}$ = 9.2 Hz, $J_{H_b-H_c}$ = 15.7 Hz, 2H, H_b), 6.66 (d, J = 9.0 Hz, 4H, H_m), 6.94 (d, J = 15.7 Hz, 2H, H_c), 7.32 (d, J = 8.9 Hz, 4H, H_o), 7.89 (d, J = 9.1 Hz, 2H, H_a). CI-MS (CH_4) 429 $(M + 1)^+$. From NOESY spectra, NOE can be seen by the cross peaks between the hydrogens H_1 and H_2 and the two hydrogens H_a. After protonation: TFA or TFA-*d* was added directly in the UV and CD cells and in the NMR tube. The yellow solution turned red. The proton data were recordered immediately after the addition of TFA (time 0), after 45 min, and after 24 h: ^{1}H-NMR (CD_3OD) (time 0) δ 1.43–1.56 (m, 2H), 1.62–1.76 (m, 2H), 1.86–2.2 (m, 2H), 2.16–2.28 (m, 2H), 3.15 (s, 12H, N-CH_3), 3.75 (m, 2H, H_1, H_2), 6.77 (d, J = 8.8 Hz, 4H, H_m), 6.83 (dd, $J_{H_b-H_a}$ = 10.8 Hz, $J_{H_b-H_c}$ = 14.6 Hz, 2H, H_b), 7.58 (d, J = 8.8 Hz, 4H, H_o), 7.74 (d, J = 14.6 Hz, 2H, H_c), 8.25 (d, J = 10.8 Hz, 2H, H_a); ^{1}H-NMR (CD_3OD) (after 45 min) (signals indicative of the unsymmetric conformation) δ 4.28 (m, 1H, H_2), 7.10 (dd, $J_{H_b''-H_a''}$ = 11.4 Hz, $J_{H_b''H_c''}$ = 14.1 Hz, 1H, $H_{b''}$), 7.83 (d, J = 14.2 Hz, 1H, $H_{c''}$), 8.29 (d, J = 11.4 Hz, 1H, $H_{a''}$), 3.75 (m, 2H, H_1, H_2), 6.68 (dd, $J_{H_b'-H_a'}$ = 10.7 Hz, $J_{H_b'-H_c'}$ = 14.5 Hz, 1H, $H_{b'}$), 7.73 (d, J = 14.8 Hz, 1H, $H_{c'}$), 8.36 (d, J = 10.7 Hz, 1H, $H_{a'}$).

3-*O*-[2,3,4,6-Tetradeoxy-2,4-bis[[[4-(dimethylamino)phenyl]methylene]-amino]-α-D-*arabino*-hexopyranosyl]-D-*chiro*-inositol (9). A solution of kasugamine (prepared from kasugamycin)[13] (16.6 mg, 0.0451 mmol) and *p*-(dimethylamino)benzaldehyde (10.1 mg, 0.0677 mmol) in anhydrous methanol (0.5 mL) was allowed to stir overnight at room temperature in the presence of anhydrous Na_2SO_4. A drop of triethylamine was added, and the mixture was passed through a short plug of silica gel with *ca.* 2:1 dichloromethane–methanol. The eluent was concentrated to afford a yellow residue (21.7 mg of the product with 30% triethylamine salt, 79% yield): ^{1}H-NMR ($CDCl_3$) δ 1.2 (m, 3H), 1.84 (m, 1H), 2.14 (m, 1H), 3.00 (s, 12H), 3.42 (m, 1H), 3.65 (t, 1H), 3.79 (m, 2H), 3.90 (d, 2H), 4.14 (s, 2H), 4.33 (m, 1H), 4.79 (br, 1H), 4.95 (d, 1H), 6.68 (t, 4H), 7.60 (m, 4H), 8.14 (s, 1H), 8.20 (s, 1H); FAB-MS (*m*-nitrobenzyl alcohol) m/z 571 $(M + 1)^+$; HRMS for $C_{30}H_{43}N_4O_7$ calcd 571.3132, found 571.3136.

3-*O*-[2,3,4,6-Tetradeoxy-2,4-bis[[(2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]-quinolizin-9-yl)methylene]amino]-α-D-*arabino*-hexopyranosyl]-D-*chiro*-inositol (10). A solution of kasugamine (20 mg, 0.065 mmol) and julolidine aldehyde (26 mg, 0.13 mmol) in anhydrous methanol (4 mL) was allowed to stir for 36 h at room temperature in the presence of anhydrous Na_2SO_4. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography (silica gel, 90:10:0.1 dichloromethane–methanol–triethylamine) to afford the product (34 mg, 78%) as a light orange solid: ^{1}H-NMR ($CDCl_3$) δ 1.25 (d, 3H), 1.90–2.05 (m, 10H), 3.00 (m, 8H), 3.50 (m, 8H), 3.58 (m, 4H), 3.75 (m, 2H), 3.90 (m, 2H), 4.2 (m, 1H), 4.35 (s, 1H), 5.00 (d, 1H), 7.16 (m, 4H), 8.05 (s, 2H), 8.12 (s, 2H); FAB-MS (*m*-nitrobenzyl alcohol) m/z 675 $(M + 1)^+$; HRMS for $C_{38}H_{50}N_4O_7$ calcd 675.3758, found 675.3784.

3-*O*-[2,3,4,6-Tetradeoxy-2,4-bis[[3-[4-(dimethylamino)phenyl]-2-propenylidene]amino]-α-D-*arabino*-hexopyranosyl]-D-*chiro*-inositol (11). A solution of kasugamine (10 mg, impure with $BaCl_2$) and excess (dimethylamino)cinnamaldehyde in anhydrous methanol (1 mL) was stirred at room temperature for 36 h. Ethyl acetate was added to precipitate the $BaCl_2$, and after filtration, the filtrate was concentrated and the residue was purified by flash chromatography (silica gel, 85:15:0.1 CH_2Cl_2–hexanes–Et_3N) to afford **11** as a yellow-orange solid (4.1 mg): R_f 0.3 (silica gel, 4:1 CH_2Cl_2–MeOH); ^{1}H-NMR (CD_3OD) δ 1.03 (d, J = 6.4 Hz, 3H), 2.30 (m, 2H), 2.89 (s, 6H), 2.91 (s, 6H), 3.60 (m, 4H), 3.72 (m, 1H), 3.82 (m, 2H), 4.31 (m, 1H), 4.92 (s, 1H), 6.63 (m, 7H), 6.98 (dd, J = 12.9 Hz, 1H), 7.31 (m, 3H), 7.42 (d, J = 9 Hz, 1H), 7.96 (d, J = 12 Hz, 1H), 8.06 (d, J = 12 Hz, 1H).

Methyl 2,3,6-Trideoxy-3-[[3-[4-(dimethylamino)phenyl]-2-propenylidene]amino-α-L-*arabino*-hexopyranoside (12). A solution of methyl α-L-acosaminide hydrochloride (2.0 mg, 0.010 mmol) and *p*-(dimethylamino)-cinnamaldehyde (1.8 mg, 0.010 mmol) in anhydrous methanol (0.5 mL) was refluxed for 2.5 h in the presence of anhydrous sodium sulfate. The mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane, and by precipitation with hexane, the pure Schiff base was obtained as its hydrochloride salt (3.4 mg, 96%): ^{1}H-NMR ($CDCl_3$, in the presence of Et_3N) δ 1.31 (d, J = 6.3 Hz, 3H, 5-C(CH_3)), 1.81 (dd, 1H, 2-CH), 1.95 (dt, 1H, 2-CH), 2.97 (s, 6H, $N(CH_3)_2$), 3.34 (s, 3H, 1-C(OCH_3)), 3.36 (m, 2H), 3.71 (m, 1H, 5-CH), 4.13 (bs, 1H, OH), 4.72 (d, J = 2.9 Hz, 1H, 1-CH), 6.63 (d, J = 8.9 Hz, 2H, *m*-Ar), 6.65 (dd, J = 8.9 and 15.8 Hz, 1H, olefinic), 6.81 (d, J = 15.8 Hz, 1H, olefinic), 7.32 (d, J = 8.9 Hz, 2H, *o*-Ar), 8.01 (d, J = 8.9 Hz, 1H, olefinic); CI-MS (CH_4) m/z 319 $(M + 1)^+$; FAB-HRMS for $C_{18}H_{27}N_2O_3$ calcd 319.2021, found 319.2022.

Methyl 2,3,6-Trideoxy-3-[[3-[4-(dimethylamino)phenyl]-2-propenylidene]amino]-4-[5-(4-methoxyphenyl)-2,4-pentadienoate]-α-L-*arabino*-hexopyranoside (13). A solution of Schiff base hydrochloride (3.4 mg, 0.010 mmol), 1-(1-oxo-5-(4-methoxyphenyl)-2,4-pentadienyl)-1*H*-1,2,4-triazole (5.6 mg, 0.020 mmol), and DBU (3.0 mg, 0.020 mmol) in anhydrous dichloromethane (2 mL) was refluxed for 45 min. Flash chromatography (silica gel, 50% ethyl acetate–hexane, *ca.* 0.1% Et_3N) of the reaction mixture afforded the bis-derivative as a yellow solid (2.2 mg, 43%): ^{1}H-NMR ($CDCl_3$, in the presence of Et_3N) δ 1.19 (d, J = 6.2 Hz, 3H, 5-C(CH_3)), 1.95 (dd, 1H, 2-CH), 2.12 (dt, 1H, 2-CH), 2.92 (s, 6H, $N(CH_3)_2$), 3.35 (s, 3H, 1-C(OCH_3)), 3.58 (m, 1H, 5-CH), 3.77 (s, 3H, *p*-Ar-OCH_3), 3.85 (m, 1H, 3-CH), 4.78 (d, J = 3.2 Hz, 1H, 1-CH), 4.89 (t, J = 9.5 Hz, 1H, 4-CH), 5.81 (d, J = 15.2 Hz, 1H, olefinic), 6.57 (d, J = 8.9, 2H, *m*-Ar), 6.62 (dd, J = 8.8, 15.8 Hz, 1H, olefinic), 6.64 (dd,

J = 10.0, 15.8 Hz, 1H, olefinic), 6.76 (d, J = 15.8 Hz, 1H, olefinic), 6.77 (d, J = 15.8 Hz, 1H, olefinic), 6.81 (d, J = 8.7 Hz, 2H, *m*-Ar), 7.25 (d, J = 8.9 Hz, 2H, *o*-Ar), 7.32 (d, J = 8.7 Hz, 2H, *o*-Ar overlapping dd, J = 10.0, 15.2 Hz, 1H, olefinic), 7.91 (d, J = 8.8 Hz, 1H, olefinic); CI-MS (CH_4) m/z 505 $(M + 1)^+$; FAB-HRMS for $C_{30}H_{37}N_2O_5$ calcd 505.2702, found 505.2713.

Methyl 2,3,6-Trideoxy-3-[[3-[4-(dimethylamino)phenyl]-2-propenylidene]amino]-β-L-*lyxo*-hexopyranoside (14). A solution of methyl β-L-daunosaminide hydrochloride (2.0 mg, 0.010 mmol) and *p*-(dimethylamino)cinnamaldehyde (2.0 mg, 0.011 mmol) in anhydrous methanol (1 mL) was refluxed for 4 h in the presence of anhydrous Na_2SO_4. The mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane, and by precipitation with hexane, the Schiff base was obtained as its hydrochloride salt (3.4 mg, 15% impure with *p*-(dimethylamino)cinnamaldehyde): ^{1}H-NMR (CD_3OD, in the presence of Et_3N) δ 1.29 (d, J = 6.8 Hz, 3H, 5-C(CH_3)), 2.99 (s, 6H, N(CH_3)$_2$), 3.48 (s, 3H, 1-C(OCH_3)), 6.72 (d, J = 8.8 Hz, 2H, *m*-Ar), 6.78 (dd, J = 9.0, 15.7 Hz, 1H, olefinic), 7.03 (d, J = 15.7 Hz, 1H, olefinic), 7.40 (d, J = 8.7 Hz, 2H, *o*-Ar), 8.09 (d, J = 9.0 Hz, 1H, olefinic); CI-MS (NH_3) m/z 319 $(M + 1)^+$.

Methyl 2,3,6-Trideoxy-3-[[3-[4-(dimethylamino)phenyl]-2-propenylidene]amino]-4-[5-(4-methoxyphenyl)-2,4-pentadienoate]-β-L-*lyxo*-hexopyranoside (15). A solution of Schiff base hydrochloride (3.4 mg, 85% purity, 0.009 mmol), 1-(1-oxo-5-(4-methoxyphenyl)-2,4-pentadienyl)-1*H*-1,2,4-triazole (5.6 mg, 0.020 mmol), and DBU (3.0 mg, 0.020 mmol) in anhydrous dichloromethane (1 mL) was refluxed for 4 h. Flash chromatography (silica gel, 35% hexane in ethyl acetate, *ca.* 0.1% Et_3N) of the reaction mixture afforded the bis-derivative as a yellow solid which was purified by HPLC (YMC-5 μ, 50% ethyl acetate–hexane, 0.1% Et_3N) to afford pure **15** as a yellow solid (2.6 mg, 59%): ^{1}H-NMR ($CDCl_3$, in the presence of Et_3N) δ 1.24 (d, J = 5.8 Hz, 3H, 5-C(CH_3)), 2.96 (s, 6H, N(CH_3)$_2$), 3.55 (s, 3H, 1-C(OCH_3)), 3.81 (s, 3H, *p*-Ar(OCH_3)), 6.60 (d, J = 15.2 Hz, 1H, olefinic), 6.62 (d, J = 8.8 Hz, 2H, *m*-Ar), 6.64 (dd, J = 8.8, 15.8 Hz, 1H, olefinic), 6.72 (dd, J = 10.6, 15.8 Hz, 1H, olefinic), 6.82 (d, J = 15.8 Hz, 2H, olefinic), 6.86 (q, J = 8.7 Hz, 2H, *m*-Ar), 7.31 (d, J = 8.8 Hz, 2H, *o*-Ar), 7.39 (d, J = 8.7 Hz, 2H, *o*-Ar), 7.46 (dd, J = 10.6, 15.2 Hz, 1H, olefinic), 8.01 (d, J = 8.8 Hz, 1H, olefinic); FAB-HRMS for $C_{30}H_{37}N_2O_5$ calcd 505.2702, found 505.2729.

5-(4-Methoxyphenyl)-2,4-pentadienoic Acid Ethyl Ester. To a solution of triethylphosphonoacetate (2.08 g, 9.26 mmol) in anhydrous THF (25 mL) under argon was added dropwise a solution of sodium bis(trimethylsilyl)amide (1.0 M in THF, 9.25 mL) over 30 min at −78 °C. The mixture was stirred at this temperature for 1 h, and (dimethylamino)-cinnamaldehyde (1.00 g, 6.17 mmol) was added under argon. The resulting mixture was stirred at room temperature under argon for 3 h, after which the reaction mixture was cooled to −78 °C and quenched by addition of a saturated solution of NH_4Cl (3 mL). The reaction mixture was stirred for 20 min at this temperature and then extracted with ether. The ether extracts were washed with brine, dried over sodium sulfate, and purified by flash chromatography (silica gel, 10% ethyl acetate–hexane) to afford the ester as white crystals (1.3 g, 91%): ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, J = 7.2 Hz, 2H, ester-CH_3), 3.80 (s, 3H, *p*-CH_3O-Ar), 4.19 (q, J = 7.2 Hz, 2H, ester-CH_2), 5.94 (d, J = 15.3 Hz, 1H, olefinic), 6.75 (dd, J = 10.7 and 15.5 Hz, 1H, olefinic), 6.86 (d, J = 15.5 Hz, 1H, olefinic), 6.89 (d, J = 8.7 Hz, 2H, *m*-Ar), 7.42 (d, J = 8.7 Hz, 2H, *o*-Ar), 7.44 (dd, J = 10.7, 15.3 Hz, 1H, olefinic).

5-(4-Methoxyphenyl)-2,4-pentadienoic Acid. 5-(4-Methoxyphenyl)-2,4-pentadienoic acid ethyl ester (1.17 g, 5.04 mmol) was dissolved in THF (20 mL), and aqueous NaOH (1 M, 7 mL) and methanol (4 mL) were added. The mixture was stirred at room temperature for 5 h, then THF and methanol were evaporated under reduced pressure. Water (10 mL) was added, and the product was isolated as a yellow powder (1.03 g, 99%) by precipitation with 1 N aqueous HCl: ^{1}H-NMR ($CDCl_3$) δ 3.74 (s, 3H, *p*-CH_3O-Ar), 5.83 (d, J = 15.1 Hz, 1H, olefinic), 6.67 (dd, J = 10.7, 15.5 Hz, 1H, olefinic), 6.73 (d, J = 15.5 Hz, 1H, olefinic), 6.79 (d, J = 8.7 Hz, 2H, *m*-Ar), 7.32 (d, J = 8.7 Hz, 2H, *o*-Ar), 7.34 (dd, J = 10.7, 15.1 Hz, 1H, olefinic).

1-[1-Oxo-5-(4-methoxyphenyl)-2,4-pentadienyl]-1*H*-1,2,4-triazole. To a solution of 5-(4-methoxyphenyl)-2,4-pentadienoic acid (1.00 g, 4.90 mmol) in anhydrous THF (25 mL) was added 1,1′-carbonylbis(1,2,4-triazole) (1.21 g, 7.39 mmol) under argon. The resulting clear solution was stirred at room temperature overnight in the dark. The bright yellow precipitate that formed was removed by filtration and washed with acetone to afford the product as a yellow crystals (362 mg, 30%). The crystals were further purified by flash chromatography (silica gel, 20% ethyl acetate–hexane): ^{1}H-NMR ($CDCl_3$) δ 3.02 (s, 3H, *p*-CH_3O-Ar), 6.89 (d, J = 8.8 Hz, 2H, *m*-Ar), 6.93 (dd, J = 11.1, 15.4 Hz, 1H, olefinic), 7.06 (d, J = 15.4 Hz, 1H, olefinic), 7.15 (d, J = 15.1 Hz, 1H, olefinic), 7.45 (d, J = 8.8 Hz, 2H, *o*-Ar), 7.85 (dd, J = 11.1, 15.1 Hz, 1H, olefinic), 8.03 (s, 1H, triazole), 8.96 (s, 1H, triazole).

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