FLUORESCENT BITHIOPHENE CHROMOPHORES: SYNTHESIS AND APPLICATION IN CD EXCITON CHIRALITY STUDIES

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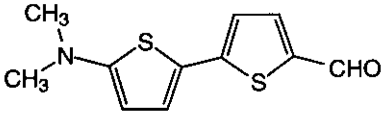
Abstract-Bithiophene chromophores were synthesized and used to derivatize NH_2 and OH groups in aminocyclohexane, (1*R*, 2*R*)-diaminocyclohexane, (1*R*, 2*R*)-*trans*-1,2-cyclohexanediol and methyl L-acosamidine for their application in the exciton chirality method. Schiff base, ester and amide derivatives were generated in good yields and were found to exhibit exciton-split CD curves. Besides their absorption at long wavelengths (red-shifted) in the visible range, the bithiophene derivatives showed fluorescence and solvatochromic properties.

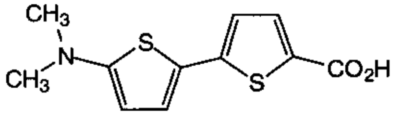
We dedicate this paper to the memory of Prof. Shun-ichi Yamada

INTRODUCTION

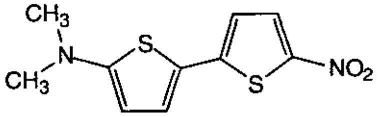
The circular dichroic (CD) exciton chirality method has proved to be very useful for determining the absolute configurations of chiral molecules, especially on a microscale level.[1,2] Chiral molecules containing two or more chromophores can interact through space to give rise to bisignate CD curves, whose sign is determined by the handedness between the corresponding electric transition dipole moments. Compounds that lack appropriate chromophoric groups can still be studied using CD by introducing chromophores to the hydroxyl and amino groups via derivatization. We have been investigating new chromophores that can be used for this purpose,[3-5] and in this report we describe new bithiophene chromophores (**1a**) and (**2a**), as well as (**1b**) and (**2b**) (with pyrrolidino instead of dimethylamino group), which can be attached to hydroxyl and amino groups and which have favorable spectroscopic and geometric features for microscale CD studies.



1a



2a



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Chromophores for use in CD studies should possess several features that can give rise to strong CD signals and make interpretation of the data unambiguous. It is preferred to use "red-shifted" chromophores that absorb at long wavelengths (>310 nm), because derivatized natural products can often contain chromophoric groups that can interact with the appended chromophores absorbing at shorter wavelengths and complicate the CD analysis.[2-5] Strongly absorbing chromophores (large molar absorptivity, ε) are desired, because CD exciton interaction (the intensity expressed as an *A* value) increases with ε.[1,2] And it is necessary to use chromophores with well defined direction of the electric transition moments that give rise to the red-shifted absorption, because the dihedral angle between these moments determines the sign of the CD exciton couplet. When this angle is positive, a positive exciton CD couplet is observed (positive Cotton effect at longer wavelengths and negative Cotton effect at shorter wavelengths). A negative angle between the transition moments give rise to a negative CD couplet. And lastly, the chromophoric groups that are linked to hydroxyl and amino groups should adopt preferred conformations at the linkage sites (ester, amide, and Schiff base bonds) (*vide infra*). The bithiophene chromophores (**1a,b** and **2a,b**) were designed based on a related compound, 5-dimethylamino-5'-nitro-2,2'-bithiophene (**3**),[6] which was reported to possess many of these desirable properties. This chromophore shows strong absorption (ε 25,000) in the visible range (466-597 nm) and possesses the charge transfer transition, which gives rise to a red-shifted absorption, that lies along the length of the bithiophene chromophore between the terminal electron donating amino and the electron accepting nitro groups.

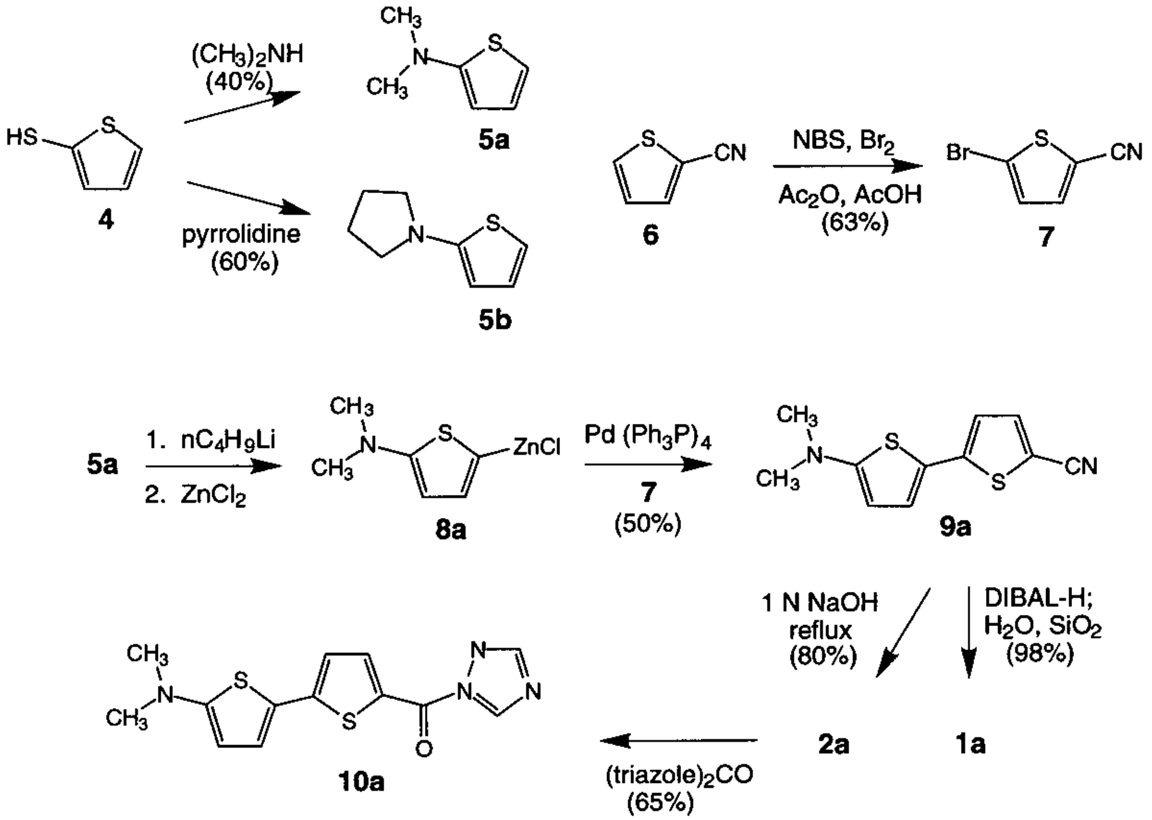
RESULTS AND DISCUSSION

Bithiophene Chromophores

The bithiophene aldehyde (**1a,b**) for derivatizing primary amines, and the carboxylic acid (**2a,b**) for linkage to hydroxyl and amino groups were prepared readily as shown in Scheme 1. The aminothiophenes (**5a,b**) were prepared from 2-mercaptothiophene (**4**) and the corresponding amine,[7] and the bromination of 2-cyanothiophene (**6**) to intermediate (**7**) was achieved under the acidic condition shown. These components were linked together at the bithiophene bond via a palladium mediated coupling of the zinc species (**8a,b**)[8] and the halide (**7**). The resulting nitriles (**9a,b**) were reduced with Dibal-H and hydrolyzed to the aldehydes (**1a,b**) or were hydrolyzed to the carboxylic acids (**2a,b**). The acid (**2a**) was converted to the activated acylating reagent (**10a**) using carbony ditriazole.[9]

The new bithiophene chromophores were in fact observed to be "red-shifted" and strongly absorbing. Aldehyde (**1a**) absorbed in the visible range at 445 nm (ε 26,500) in acetonitrile and aldehyde (**1b**), with its more strongly electron donating cyclic amino group, at 459 nm (ε 29,800). These aldehydes could be reacted with primary amines to form Schiff base derivatives. With cyclohexylamine, derivatives (**11a**) (409 nm, ε 21,900) and (**11b**) (422 nm, ε 28,000) were formed, and addition of trifluoroacetic acid (TFA) resulted in the protonated Schiff base derivatives (**11a'**) (559 nm, ε 44,900) and (**11b'**) (572 nm, ε 60,400) with enhanced absorption at much longer wavelengths (Scheme 2). The ester and amide derivatives showed favorable absorptions as well. The methyl ester (**12a**) and cyclohexylamide (**13a**) were prepared by treating the alcohol and amine with (**10a**) in the presence of DBU base. The ester (**12a**) (412 nm, ε 22000) and amide (**13a**) (394 nm, ε 15,400) also showed distinct absorptions at long wavelengths.

These chromophores also adopt favorable conformation. The bithiophene rings most likely adopt the *anti* orientation as drawn (Scheme 2) as seen in the crystal structure of 5-dimethylamino-5'-nitro-2,2'-bithiophene (**3**).[6] This is also consistent with the absence of NOESY cross peak between protons (H_c and H_d) on adjacent thiophene rings.[10]

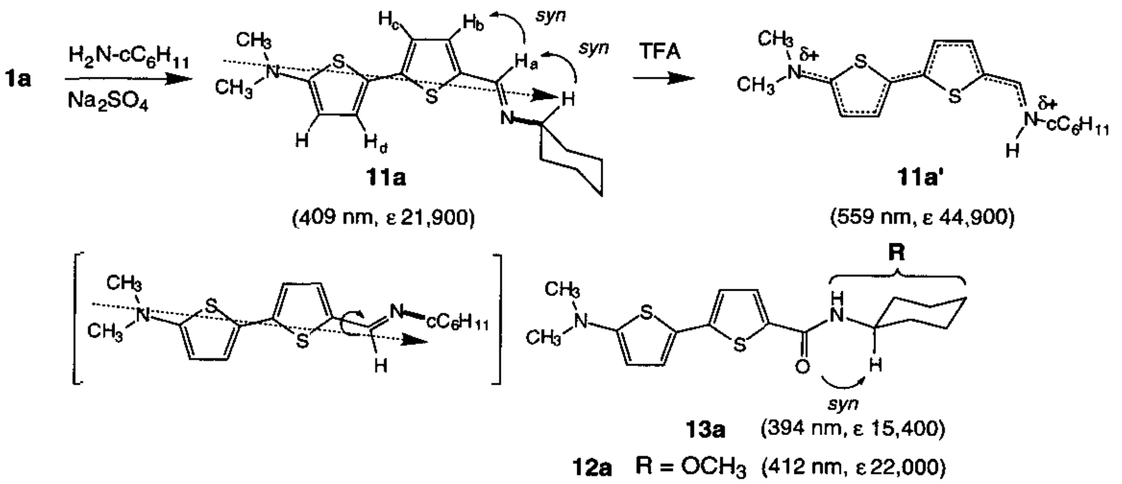


Scheme 1

The orientation of the thiophene-imine single bond as drawn is consistent with molecular mechanics calculations[11] and the weak NOESY cross peak between the imino hydrogen H_a and the adjacent thiophene hydrogen H_b.[10] However, the alternative orientation in brackets (rotation by 180° about the C-C bond) will still maintain an almost collinear alignment of the transition moment (dotted arrow) and the amino N-cHex single bond (shown bold). Thus, the electric transition moments lie along the long axis of the chromophores and are parallel to the hydroxyl C-O bond and amino C-N bond (Scheme 2). The chromophores also adopt preferred conformations at the linkage sites, for example, the H_a of the Schiff base, and the ester and amide carbonyls should be *syn* with respect to the methine hydrogens at the stereogenic centers as depicted on Scheme 2. With these orientations in a system with two attached chromophores, the angle between the transition moments correctly represents the chiral sense of twist between the derivatized hydroxyl and amino groups at the stereogenic centers of the template molecule.

Exciton Coupled CD Spectra

With their favorable chromophoric and geometric features, it was expected that the bithiophene chromophores would show strong exciton split CD spectra with signs that correctly reflect the relative orientation of the hydroxyl and amino groups in the derivatized chiral molecules. This was tested with chiral template molecules with vicinal hydroxyl and/or amino groups and known absolute configuration and conformation.

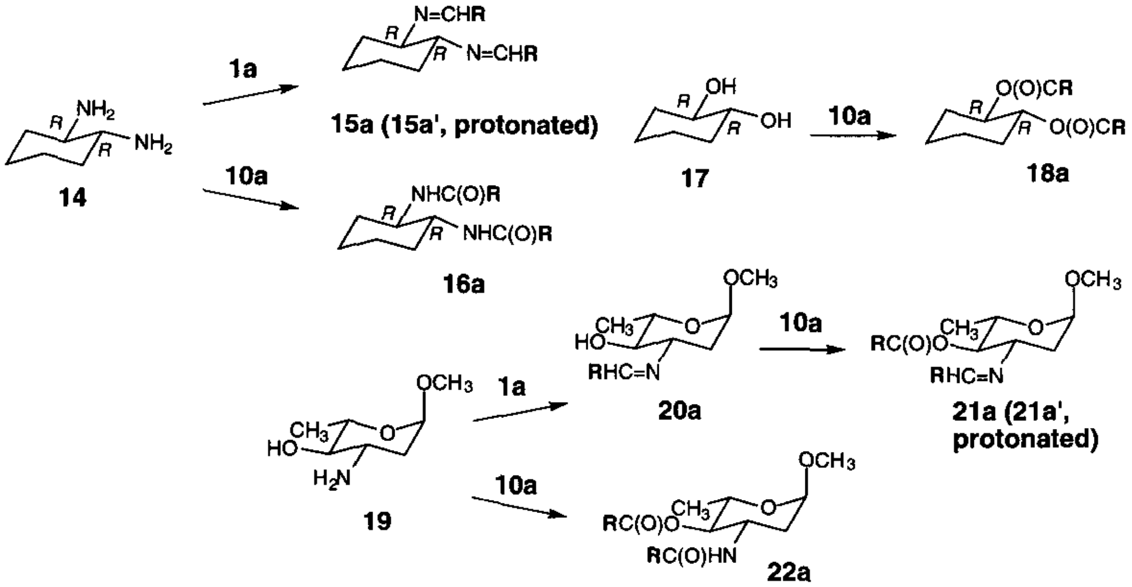


Scheme 2

The derivatization was performed as summarized in Scheme 3. (1*R*, 2*R*)-Diaminocyclohexane (**14**) places its amino groups in the thermodynamically preferred equatorial positions with a negative dihedral angle between the two C-N bonds. The bithiophene chromophores linked to them should also be equatorial with a negative dihedral angle between the transition moments to give rise to a negative CD bisignate curve (negative and position Cotton effects at longer and shorter wavelengths, respectively). Indeed, both the neutral bis-Schiff base derivative (**15a**) [UV: 410 nm (ε 34,000)] and the protonated bis-Schiff base (**15a'**) display negative CD curves (Figure 1a). These bisignate CD curves are centered at the absorption maxima of the UV-vis spectra, and the stronger protonated Schiff base chromophore (larger ε) give rise to a stronger CD curve. The positive and negative CD Cotton effects arise from mixing of the electric transitions of the two chromophores that give rise to two transition states, one with lower and one with higher energies. It is interesting to note that the UV-vis spectrum of the protonated Schiff base (**15a'**) was resolved into two bands at 552 nm (ε 67,000) and 614 nm (ε 44,000). There are only a few examples where such exciton split bands arising from the mixing of the excited states are observed in the UV-vis as well as in the CD spectra .[4,12]

The bis-amide derivative (**16a**) prepared from the same diamine similarly displayed negative CD curve. The bis-ester derivative (**18a**) prepared from (1*R*,2*R*)-*trans*-1,2-cyclohexanediol (**17**) with the same configuration as the diamine (**14**) also gave negative CD curve, which was centered at the UV-vis band at 414 nm (ε 47,000) (Figure 1b).

The exciton CD interaction between two different chromophores was studied using methyl L-acosamidine (**19**) as the template molecule. This has vicinal amino and hydroxyl groups placed at equatorial positions of the pyranose ring and with a positive dihedral angle between them. The mixed Schiff base-ester derivative (**21a**) was prepared by first reacting the amino group with the aldehyde (**1a**) without protecting the hydroxyl group, then reacting the hydroxyl group with (**10a**). The interaction of the two different chromophores gave rise to the expected positive CD curve (Figure 2a).



Scheme 3

Addition of TFA formed the protonated Schiff base derivative (**21a'**) with the positive CD band shifted to the red, and which retained the positive CD bisignate curve. The neutral Schiff-base derivative (**21a**) showed a stronger CD interaction (*A* =+82) compared with the protonated Schiff-base (**21a'**) (*A* =+30), because exciton CD interactions are stronger when the interacting chromophores have transitions of similar energies (wavelengths). The amide-ester derivative (**22a**) was prepared from methyl α-L-acosamidine in one step using reagent (**10a**). Again, the CD curve was positive as expected from the orientation of the amino and hydroxyl groups, with the couplet centered at the UV-vis band at 406 nm (ε 40,000) (Figure 2b).

The preparation of the chromophores and their derivatives for UV-vis and CD studies were straightforward and is described in the Experimental Section. It should be noted, also, that the bithiophene chromophores as a dilute solution were stable in absence of light, while they were rather sensitive to exposure to light and showed weakening in their UV-vis and CD signals with repeated measurements. Thus, samples should be prepared with limited exposure to light and then used for a single measurement, if accurate signal intensities are desired.

Fluorescence Properties

In addition to strong absorption at long wavelengths, the bithiophene chromophores possessed additional chromophoric properties that may make them useful for other application besides exciton CD. The chromophores (**1a,b** and **2a,b**) as well as their Schiff base, ester and amide derivatives displayed strong fluorescence. The fluorescence of bithiophenealdehyde (**1a**) was compared with that of two strongly fluorescent compounds, naphthalene and methyl 2-anthroate. At the same concentration (2×10^{-5} M) in hexanes, the intensity of the fluorescence peak for (**1a**) (Ex = 395 nm, Em = 463 nm) was twelve-fold larger than that of naphthalene (Ex = 268 nm, Em = 324 nm) and six-fold greater than for methyl 2-anthroate (Ex = 290 nm, Em = 420 nm).

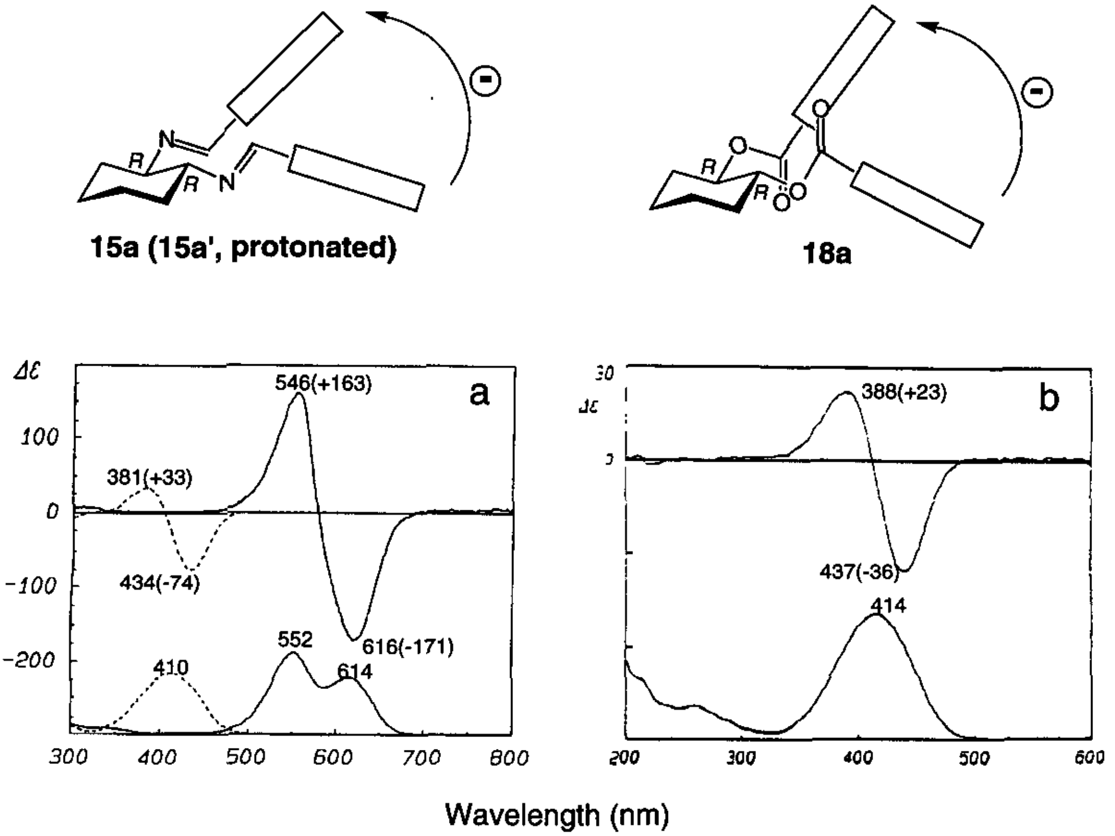


Figure 1: CD spectra (top) and UV-vis spectra (bottom) in acetonitrile: (**a**) neutral form (**15a**) (dashed line) and protonated form (**15a'**) (solid line); (**b**) bisester (**18a**). The λmax (Δε) values are given. The rectangles represent the dimethylaminobithiophene chromophore.

Fluorescence is a desirable feature when studying samples on a microscale, because higher sensitivity of detection has been observed using a fluorescence instead of an UV-vis detector.[13] The relative sensitivities for these two modes of detection were compared using the bisester bithiophene derivative (**18a**). This sample was injected onto a normal phase silica gel HPLC column, elution with 15% ethyl acetate-hexane, 1 mL/min, peak elution at 10 min) and detected either by UV-vis (at 398 nm) or by fluorescence (Ex = 398 nm, Em = 450 nm). With UV-vis detection, 10 pmol of sample was observable with signal to noise ratio (S/N) of five. With fluorescence detection, the sensitivity was increased 25-fold with detection of 0.4 pmol sample at the same S/N. The Schiff base derivative (**11b**) in acetonitrile displayed fluorescence (Ex = 422 nm, Em = 518 nm) with a linear relationship in the peak height of the eluted peak to concentration. Using a fluorescence spectrometer, a fluorescence signal was observable for a solutions of (**11b**) in acetonitrile down to 10^{-8} M. The protonated Schiff base (**11b'**), on the other hand, exhibited virtually no fluorescence.

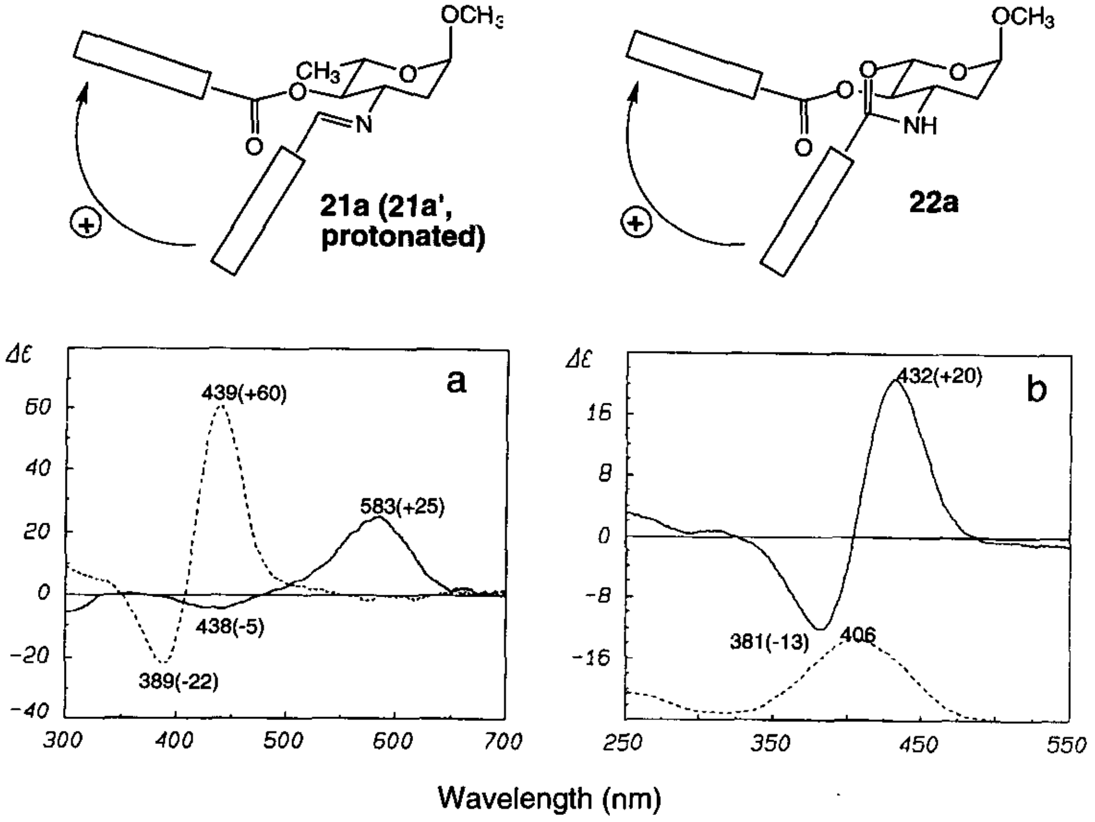


Figure 2: CD and UV-vis spectra in acetonitrile: (**a**) CD of neutral form (**21a**) (dashed line) and protonated form (**21a'**) (solid line); (**b**) CD of (**22a**) (solid line) and UV-vis (dashed line). The λmax (Δε) values are given. The rectangles represent the dimethylaminobithiophene chromophore.

Solvatochromic Effect

Another notable feature of the bithiophene chromophores is the rather large change in the UV-vis absorption maxima depending on the solvent used (solvatochromic effect). Recently, Lightner reported the large solvatochromic effect observed for phenoxides of p-hydroxycinnamic acid ester derivatives that are useful for CD studies.[14] For 5-dimethylamino-5'-nitro-2,2'-bithiophene (**3**), the range in absorption maxima was reported to vary more than 100 nm in the range of solvents studied.[6] There was a very good correlation between the absorption wavelengths and the known π* values of Taft, which describe the polarity and polarizability of the solvent. This allows one to use this compound as an indicator dye to determine the π* value of a solvent.[6] The solvatochromic effect with our bithiophene compounds (**1a**, **11a,b**, and **18a**) were studied by measuring the absorption and fluorescence in different solvents (Table 1). A range in absorptions (*ca.* 20-40 nm) was observed in different solvents, with more polar solvents giving absorption maxima at longer wavelengths. As reported previously for **3**, our compounds also showed good correlation between absorption maxima and the Taft constant π*. The solvatochromic effect was also observed with fluorescence (*ca.* 50-100 nm change), with the fluorescence bands occurring at longer wavelengths and with weaker intensities in more polar solvents.

Table 1: Absorption (UV) and emission (Fluor) maxima values (nm) and intensities in parenthesis (arbitrary units) for compounds (**1a**, **11a**, **11b** and **18a**) at the indicated concentrations in different solvents.

	1a (10^{-5}M)		**11a** (3×10^{-4}M)		**11b** (2×10^{-4}M)		**18a** (2×10^{-7}M)	
Solvent	UV-λ*max*	Fluor-λ*max*	UV-λ*max*	Fluor-λ*max*	UV-λ*max*	Fluor-λ*max*	UV-λ*max*	Fluor-λ*max* (intensity)
hexane	421	464	394	468	408	476	394	448 (345), 464 (336)
ethyl ether	430	500	400	495	413	486	405	478 (402)
benzene	442	507	410	503	420	503	412	482 (329)
acetone	443	546	408	512	422	508	412	513 (58)
acetonitrile	448	554	409	524	422	518	415	524 (31)
dichloromethane	451	540	410	520	426	550	425	501 (142)
methanol	452	555	415	527	430	520	413	534 (9)
DMSO	458	559	417	530	430	530	424	531 (20)

This study showed that the bithiophene chromophores with electron donating and accepting groups can be readily prepared and can be used to derivatize and study chiral molecules containing hydroxyl and amino groups by CD. The CD data accurately reflected the chirality of the derivatized amino and hydroxyl groups. These chromophores exhibit absorption at long wavelengths (red shifted) in the visible range. They can be detected at very high sensitivity using fluorescence and also show an interesting and useful solvatochromic effect.

EXPERIMENTAL SECTION

General Procedures. Solvents were reagent grade. Anhydrous solvents were dried and distilled (THF from Na / benzophenone; CH_2Cl_2 from CaH_2). The reagents from commercial suppliers were used without further purification, unless otherwise noted. Moisture sensitive reactions were done in flame-dried glassware under argon. Thin-layer chromatography was done using Analtech Silica Gel GHLF (250 nm thick) and flash chromatography with ICN silica gel (32-63 mesh). HPLC purification and analysis were done using a Hypersil Silica 5 μm column (4.6 x 150 mm) on a Perkin-Elmer Series 4 Liquid Chromatograph 7500 Data Station connected to a Hewlett Packard 1046A Programmable Fluorescence Detector and a Rainin Dynamax model UV-D detector. ^{1}H-NMR spectra were obtained on Varian VXR instruments and are reported in parts per million (δ) relative to $CHCl_3$ (7.27 ppm) as an internal standard. FAB mass spectra were measured on a JEOL JMS-DX303 HF mass spectrometer using glycerol matrix and Xe ionizing gas; CI and EI spectra were obtained with a NERMAG R10-10 spectrometer. UV-VIS and CD spectra were recorded on a Perkin-Elmer Lambda 4B UV/VIS spectrophotometer and JASCO J-720 spectropolarimeter. The fluorescence spectra were recorded on a Fluoromax 2 spectrofluorometer.

2-Dimethylaminothiophene (5a). A mixture of 2-mercaptothiophene (4) (1.79 g, 15.4 mmol) and toluene (5 mL) that had been saturated with dimethylamine gas was heated at 100 °C for 30 min. During this time, the solid that formed on the condenser was periodically scraped back into the reaction flask, and more dimethylamine gas was bubbled through the reaction mixture. Toluene was removed by distillation at aspirator pressure, and the product was collected as a yellow liquid (0.785 g, 40%) after distillation under high vacuum. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.89 (s, 6H, C*H*$_3$), 6.10 (dd, *J* = 3.8, 1.5 Hz, 1H, H-3), 6.47 (dd, *J* = 5.5, 1.4 Hz, 1H, H-5), 6.76 (dd, *J* = 5.5, 3.8 Hz, 1H, H-4). CI-MS (CH_4) *m/z* 128 (M+1, 100%), 156 (18%).

2-Pyrrolidinothiophene (5b). To a solution of 2-mercaptothiophene (**4**) (1.02 g, 8.80 mmol) in toluene (7 mL) was added pyrrolidine (0.73 mL, 8.80 mmol). The mixture was heated 14 min at reflux until evolution of H_2S gas had seized. Toluene was removed by distillation at aspirator pressure, and the residue was distilled under high vacuum to yield the desired product as a yellow liquid (0.807 g, 60%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.00 (m, 4H, C*H*$_2$), 3.24 (m, 4H, N-C*H*$_2$), 5.73 (d, *J* = 3.6 Hz, 1H, H-3), 6.36 (d, *J* = 5.4 Hz, 1H, H-5), 6.77 (dd, *J* = 3.9, 5.2 Hz, 1H, H-4). CI-MS (CH_4) *m/z* 154 (M+1, 100%), 182 (23%).

5-Bromothiophene-2-carbonitrile (7). To a mixture of 2-cyanothiophene (**6**) (2.22 g, 0.0203 mmol) and *N*-bromosuccinimide (3.62 g, 0.0203 mmol) in acetic anhydride (8.30 g, 0.0813 mmol) and glacial acetic acid (1.22 g, 0.0203 mmol) was added bromine (1.05 mL, 0.0203 mmol). The mixture was stirred 3 h during which time the *N*-bromosuccinimide dissolved completely and precipitate subsequently formed. The reaction mixture was poured into ice water containing sodium bisulfite, and the mixture was stirred overnight. The aqueous mixture was extracted with dichloromethane, and the organic layer was washed with water and sat'd $NaHCO_3$ then dried over anhydrous $MgSO_4$. After filtration and rotary evaporation, the residue was purified by flash chromatography (silica gel, 3% ethyl acetate-hexane) to afford the bromide as a pale yellow liquid (2.40 g, 63%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.08 (d, *J* = 4.0 Hz, 1H), 7.38 (d, *J* = 4.0 Hz, 1H). CI-MS (CH_4) *m/z* 188 (M+1, 100%), 190 (M+3, 90%).

5-Dimethylamino-5'-cyano-2,2'-bithiophene (9a). To a solution of 2-dimethylaminothiophene (**5a**) (254 mg, 2.00 mmol) in tetrahydrofuran (2 mL) cooled over an ice bath was added n-butyllithium (1.6 *M* in hexane, 1.62 mL, 2.60 mmol) over 7 min. After 10 min, the ice bath was removed, and the solution was stirred 2 h at rt. The mixture was transferred *via* cannula to a solution of anhydrous $ZnCl_2$ (299 mg, 2.20 mmol) in tetrahydrofuran (2 mL), and the mixture was stirred 1 h at rt. Palladium tetrakis(triphenylphosphine) (115 mg, 0.10 mmol) and then 5-bromothiophene-2-carbonitrile (**7**) (0.233 mL, 2.00 mmol) were added, and the mixture was stirred 18 h at rt. The mixture was diluted with dichloromethane, washed with sat'd NH_4Cl, and the organic layer was dried over anhydrous $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (silica gel, 10:1 to 5:1 hexane-ethyl acetate) to afford the bithiophene derivative (234 mg, 50%) as an orange-brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.98 (s, 6H, C*H*$_3$), 5.78 (d, *J* = 4 Hz, 1H, thioph), 6.80 (d, *J* = 4 Hz, 1H, thioph), 7.01 (d, *J* = 4 Hz, 1H, thioph), 7.42 (d, *J* = 4 Hz, 1H, thioph). EI-MS *m/z* 234 (M^+, 100%). EI-HRMS for $C_{11}H_{10}N_2S_2$ calcd 234.0285, found 234.0300.

5-Cyano-5'-pyrrolidino-2,2'-bithiophene (9b). To a solution of 2-pyrrolidinothiophene (**5b**) (236 mg, 1.54 mmol) in tetrahydrofuran (2 mL) cooled over an ice bath was added n-butyllithium (1.6 *M* in hexane, 1.25 mL, 2.00 mmol) over 5 min. After 10 min, the ice bath was removed, and the solution was stirred 2 h at rt. The mixture was transferred *via* cannula to a solution of anhydrous $ZnCl_2$ (231 mg, 1.69 mmol) in tetrahydrofuran (2 mL), and the mixture was stirred 1 h at rt. Palladium tetrakis(triphenylphosphine) (89 mg, 0.08 mmol) and then 5-bromothiophene-2-carbonitrile (**7**) (0.180 mL, 1.54 mmol) were added, and the mixture was stirred 18 h at rt. The mixture was diluted with dichloromethane, washed with sat'd NH_4Cl, and the organic layer was dried over anhydrous $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (silica gel, 10:1 to 5:1 hexane-ethyl acetate) to afford the bithiophene derivative (209 mg, 52%) as a orange-brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.07 (m, 4H, CH_2), 3.32 (m, 4H, CH_2N), 5.68 (d, J = 4 Hz, 1H, thioph), 6.80 (d, J = 4 Hz, 1H, thioph), 7.04 (d, J = 4 Hz, 1H, thioph), 7.42 (d, J = 4 Hz, 1H, thioph). EI-MS *m/z* 260 (M^+, 100%). EI-HRMS for $C_{13}H_{12}N_2S_2$ calcd 260.0442, found 260.0455.

5-Dimethylamino-5'-formyl-2,2'-bithiophene (1a). To a solution of (**9a**) (102 mg, 0.437 mmol) in dichloromethane (10 mL) at -78 °C was added DIBAL-H (1 *M* in hexane, 0.524 mL, 0.524 mmol) over 4 min. After 30 min, several drops of water and silica gel was added to the reaction mixture. The cooling bath was removed, and the slurry was stirred 30 min at rt. The mixture was passed through a pad of silica gel, eluting with ethyl acetate, and the filtrate was evaporated to leave a orange-brown solid, which was essentially pure aldehyde (102 mg, 98%). The product was recrystallized from ethyl acetate and hexane to afford reddish brown amorphous solid. This was recrystallized from ethyl acetate and hexane. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.01 (s, 6H, CH_3), 5.82 (d, J = 4 Hz, 1H, thioph), 6.96 (d, J = 4 Hz, 1H, thioph), 7.14 (d, J = 4 Hz, 1H, thioph), 7.59 (d, J = 4 Hz, 1H, thioph), 9.78 (s, 1H, CHO). EI-MS *m/z* 237 (M^+, 100%). EI-HRMS for $C_{11}H_{11}NOS_2$ calcd 237.0282, found 237.0292.

5-Formyl-5'-pyrrolidino-2,2'-bithiophene (1b). To a solution of (**9b**) (158 mg, 0.611 mmol) in dichloromethane (2 mL) at -78 °C was added DIBAL-H (1 *M* in hexane, 0.733 mL, 0.733 mmol) over 10 min. After 30 min, several drops of water and silica gel was added to the reaction mixture. The cooling bath was removed, and the slurry was stirred 30 min at rt. The mixture was passed through a pad of silica gel, eluting with ethyl acetate, and the filtrate was evaporated to leave a orange-red solid, which was essentially pure aldehyde (156 mg, 97%). This was recrystallized from ethyl acetate and hexane. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.08 (t, 4H, CH_2), 3.34 (t, 4H, CH_2N), 5.71 (d, J = 4 Hz, 1H, thioph), 6.92 (d, J = 4 Hz, 1H, thioph), 7.16 (d, J = 4 Hz, 1H, thioph), 7.58 (d, J = 4 Hz, 1H, thioph), 9.74 (s, 1H, CHO). EI-MS *m/z* 263 (M^+, 100%). EI-HRMS for $C_{13}H_{13}NOS_2$ calcd 263.0439, found 263.0433.

5'-Dimethylamino-2,2'-bithiophene-5-carboxylic acid (2a). A suspension of (**9a**) (72 mg, 0.31 mmol) in aqueous NaOH (1*N*, 20 mL) and dioxane (4 mL) was heated at reflux 4 h until the solid dissolved. After cooling, the mixture was acidified with concentrated HCl, and the resulting precipitate was filtered and washed with water. The filtrate was extracted with dichloromethane, dried over anhydrous Na_2SO_4 and evaporated. The residue was combined with the filtered solid and purified by flash chromatography (silica gel, 3-10% methanol-dichloromethane) to yield the acid (63 mg, 80%). This was recrystallized from methanol. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.00 (s, 6H, CH_3), 5.86 (d, J = 4 Hz, 1H, thioph), 6.91 (d, J = 4 Hz, 1H, thioph), 7.06 (d, J = 4 Hz, 1H, thioph), 7.70 (d, J = 4 Hz, 1H, thioph). EI-MS *m/z* 253 (M^+, 100%). EI-HRMS for $C_{11}H_{11}NO_2S_2$ calcd 253.0231, found 253.0229.

5'-(1-Pyrrolidinyl)[2,2'-bithiophene]-5-carboxylic acid (2b). A suspension of (**9b**) (40 mg, 0.15 mmol) in aqueous NaOH (1*N*, 20 mL) and dioxane (6 mL) was heated at reflux 6 h until the solid dissolved. After cooling, the mixture was acidified with concentrated HCl, and the resulting precipitate was filtered and washed with water. The filtrate was extracted with dichloromethane, dried over anhydrous Na_2SO_4 and evaporated. The residue was combined with the filtered solid and purified by flash chromatography (silica gel, 3-10% methanol-dichloromethane) to yield the acid as a solid (34 mg, 78%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.07 (m, 4H, CH_2), 3.32 (m, 4H, CH_2), 5.69 (d, J = 4 Hz, 1H, thioph), 6.86 (d, J = 4 Hz, 1H, thioph), 7.08 (d, J = 4 Hz, 1H, thioph), 7.68 (d, J = 4 Hz, 1H, thioph). EI-MS *m/z* 279 (M^+, 100%). EI-HRMS for $C_{13}H_{13}NO_2S_2$ calcd 279.0388, found 279.0382.

5-Carboxyl-5'-dimethylamino-2,2'-bithiophene triazole amide (10a). The acid (**2a**) (38 mg, 0.15 mmol) and 1,1'-carbonyldi(1,2,4-triazole) (52 mg, 0.32 mmol) in acetonitrile (4 mL) were stirred 2 days at rt. The deep red mixture was concentrated under vacuum, and the residue was purified by flash chromatography (silica gel, 2:1 hexane-ethyl acetate) to yield the triazole amide (32 mg, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.04 (s, 6H, C*H*3), 5.87 (d, *J* = 4 Hz, 1H, thioph), 7.00 (d, *J* = 4 Hz, 1H, thioph), 7.23 (d, *J* = 4 Hz, 1H, thioph), 8.13 (s, 1H, triazole), 8.40 (d, *J* = 4 Hz, 1H, thioph), 9.07 (s, 1H, triazole). EI-MS *m/z* 304 (M^+, 100%). EI-HRMS for $C_{13}H_{12}N_4OS_2$ calcd 304.0453, found 304.0445.

***N*-[[5'-(Dimethylamino)[2,2'-bithiophen]-5-yl]methylene]-cyclohexylamine (11a).** The aldehyde (**1a**) (15 mg, 0.063 mmol) and cyclohexylamine (7.2 µL, 0.063 mmol) were dissolved in 1:1 methanol-dichloromethane (0.6 mL) and stirred 3 days at rt in the presence of anhydrous Na_2SO_4. The mixture was filtered through Celite and the volatiles were removed under vacuum to yield a residue. Purification by flash chromatography (silica gel, 10:1 hexane-ethyl acetate with 1% triethylamine) furnished the Schiff base (17 mg, 85 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.2-1.8 (m, 10H, cHex), 2.96 (s, 6H, CH_3), 3.13 (m, 1H, C*HN*), 5.78 (d, *J* = 4 Hz, thioph), 6.84 (d, *J* = 4 Hz, thioph), 6.96 ((d, *J* = 4 Hz, thioph), 7.09 (d, *J* = 4 Hz, thioph), 8.29 (s, 1H, C*H*N). EI-MS *m/z* 318 (M^+, 100%). EI-HRMS for $C_{17}H_{22}N_2S_2$ calcd 318.1224, found 318.1219.

***N*-[5'-(1-Pyrrolidinyl)[2,2'-bithiophen]-5-yl]methylene]cyclohexylamine (11b).** Prepared as for (**11a**). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.32 (m, 4H, cHex), 1.58 (dq, 2H, cHex), 1.74 (d, 2H, cHex), 1.82 (dt, 2H, cHex), 2.04 (m, 4H, pyrrol), 3.12 (m, 1H, C*HN*), 3.30 (m, 4H, pyrrol), 5.65 (d, *J* = 3.9 Hz, 1H, thioph), 6.82 (d, *J* = 3.8 Hz, 1H, thioph), 6.98 (d, *J* = 4.0 Hz, 1H, thioph), 7.08 (d, *J* = 3.8 Hz, 1H, thioph), 7.27 (s, 1H, olefinic). EI-MS *m/z* 344 (M^+, 100%). EI-HRMS for $C_{19}H_{24}N_2S_2$ calcd 344.1381, found 344.1378.

5-Carboxyl-5'-dimethylamino-2,2'-bithiophene methyl ester (12a). To a solution of **10a** (0.8 mg, 0.003 mmol) in 1 mL of anhydrous methanol was added DBU (0.4 mL, 0.003 mmol) and stirred 15 minutes at rt. The solvent was evaporated, and the residue was purified by flash chromatography (silica gel, 2:1 hexane-ethyl acetate) to afford the methyl ester (0.7 mg, quant.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.9 (s, 6H, $N(Me)_2$), 3.88 (s, 3H, CH_3), 5.81 (d, *J* = 4 Hz, 1H, thioph), 6.89 (d, *J* = 4 Hz, 1H, thioph), 7.04 (d, *J* = 4 Hz, 1H, thioph), 7.65 (d, *J* = 4 Hz, 1H, thioph). CI-MS m/z 268 $(M+1)^+$. FAB-HRMS for $C_{12}H_{13}NO_2S_4$ calcd 267.0388, found 267.0393.

***N*-[5-Dimethylamino[2,2'-bithiophene]-5-carboxyl]cyclohexyl amide (13a).** To a solution of cyclohexylamine (0.38 mL, 0.0033 mmol) and **10a** (1.5 mg, 0.0049 mmol) in 0.4 mL of anhydrous dichloromethane was added DBU (0.76 mL, 0.0049 mmol), and the mixture was stirred 1 day at rt. The solvent was evaporated and the residue was purified by preparative TLC to yield the monoamide (0.93 mg, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.43 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 1.76 (d, 2H, CH_2), 2.06 (d, 2H, CH_2), 2.98 (s, 6H, $N(CH_3)_2$), 3.90 (m, 2H, C*HN*), 5.77 (d, *J* = 3.8 Hz, 2H, thioph), 5.87 (bs, 1H, N*H*), 6.87 (d, *J* = 4.0 Hz, 2H, thioph), 6.97 (d, *J* = 3.3 Hz, 2H, thioph), 7.33 (d, *J* = 3,8 Hz, 2H, thioph). CI-MS m/z 335 $(M+1)^+$. FAB-HRMS for $C_{17}H_{22}N_2OS_2$ calcd 334.1174, found 334.1164

(1*R*-*trans*)-*N*,*N'*-Bis[[5'-(dimethylamino)[2,2'-bithiophen]-5-yl]methylene]-1,2-cyclohexylamide (15a). The aldehyde (**1a**) (14.8 mg, 0.0624 mmol), (1*R*, 2*R*)-1,2-cyclohexanediamine (0.1*M* in methanol, 0.312 mL, 0.0312 mmol) and dichloromethane (0.3 mL) were combined, and then the solution was refluxed 1 h in the presence of molecular sieves. The mixture was filtered through Celite, and the volatiles were removed under vacuum to yield a residue. Purification by flash chromatography (silica gel, 5:1 hexane-ethyl acetate with 1% triethylamine) furnished the Schiff base (12.5 mg, 72%). This was recrystallized from ethyl acetate and hexane. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.48 (m, 2H, CH_2), 1.6-1.9

(m, 8H, CH_2), 2.94 (s, 6H, CH_3), 3.27 (br m, 2H, CHN), 5.77 (d, *J* = 4 Hz, 2H, thioph), 6.76 (d, *J* = 4 Hz, 2H, thioph), 6.92 (d, *J* = 4 Hz, 2H, thioph), 6.97 (d, *J* = 4 Hz, 2H, thioph), 8.15 (s, 2H, HC=N). FAB-MS *m/z* 553 (M^+). FAB-HRMS for $C_{28}H_{32}N_4S_4$ calcd 552.1510, found 552.1515.

(1*R*-*trans*)-*N*,*N'*-bis[5'-(Dimethylamino)[2,2'-bithiophene]-5-carboxyl]cyclohexylamide (16a). To (1*R*, 2*R*) *trans*-1,2-diaminocyclohexane (**14**) (0.1*M* in CH_2Cl_2, 0.08 mL, 0,008 mmol) and **10a** (4.9 mg, 0.016 mmol) in CH_2Cl_2 (1 mL) was added DBU (2.4 mL, 0.016 mmol), and the mixture was stirred one day at rt. The solvent was evaporated. The residue was purificated by TLC (silica gel, 10% methanol-dichloromethane) to afford the diamide (4 mg, 90%). ^1H-NMR (400 MHz, $CDCl_3$) δ 1.48 (m, 2H, CH_2), 1.58(m, 2H, CH_2), 1.80 (d, 2H, CH_2), 2.21 (d, 2H, CH_2), 2.96 (s, 12 H, $N(CH_3)_2$), 3.9 (br s, 2H, CH-N), 5.83 (d, *J* = 3.8 Hz, 2H, thioph), 6.61 (s, 2H, NH), 6.84 (d, *J* = 3.9 Hz, 2H, thioph), 6.96 (d, *J* = 3.7 Hz, 2H, thioph), 7.33 (d, *J* = 3.9 Hz, 2H, thioph). CI-MS *m/z* 585 $(M+1)^+$. FAB-HRMS for $C_{28}H_{32}N_4O_2S_4$ calcd 584.1408, found 584.1395.

(1*R*-*trans*)-1,2-Cyclohexanediyl bis[5'-(dimethylamino)[2,2'-bithiophene]-5-carboxylate] (18a). To (1*R*, 2*R*)-*trans*-1,2-cyclohexanediol (**17**) (0.65 mg, 0.0056 mmol) and (**10a**) (6.4 mg, 0.021 mmol) in acetonitrile (2 mL) was added DBU (3.1 μL, 0.021 mmol), and the mixture was stirred 1 day at rt. Two drops of water were added and stirred 1 h. The solvent was evaporated, and the residue was purified by preparative TLC (silica gel, 10% methanol-dichloromethane) to afford the bis-ester (3.3 mg, quant). The product was subjected to HPLC purification (5 μ, 15 % ethyl acetate-hexane) before CD/UV measurements. ^1H-NMR (400 MHz, $CDCl_3$) δ 1.48 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.80 (br d, 2H, CH_2), 2.21 (br d, 2H, CH_2), 2.95 (s, 6H, CH_3), 5.10 (m, 2H, CHO), 5.77 (d, *J* = 4 Hz, 2H, thioph), 6.80 (d, *J* = 4 Hz, 2H, thioph), 6.99 (d, *J* = 4 Hz, 2H, thioph), 7.58 (d, *J* = 4 Hz, 2H, thioph). FAB-MS *m/z* 586 (M^+). FAB-HRMS for $C_{28}H_{30}N_2O_4S_4$ calcd 586.1088, found 586.1085.

Methyl [5-dimethylamino-2,2'-bithiophene-5'-methylene-amino]-α-L-*arabino*-hexopyranoside (20a). A solution of methyl α-*L*-acosamidine hydrochloride (**19**) (1.8 mg, 0.0091 mmol) and aldehyde (**1a**) (2.3 mg, 0.0091 mmol) in anhydrous methanol (0.5 mL) was refluxed 6 h in the presence of molecular sieves and under argon atmosphere. The mixture was filtered and washed with methanol, and the solvent was evaporated under reduced pressure. The residue was purified by preparative TLC (silica gel, ethyl acetate) to afford the Schiff base (2.6 mg, 70%). This was recrystallized from ether. ^1H-NMR (400 MHz, $CDCl_3$) δ 1.36 (d, *J* = 6.2 Hz, 3H, CH_3), 1.90 (dd, 1H, 2-CH), 2.1 (dt, 1H, 2-CH), 2.98 (s, 6H, $N(CH_3)_2$), 3.4 (s, 3H, OCH_3), 3.53 (m, 1H, CH), 3.78 (m, 1H, CH), 4.79 (d, *J* = 3.3 Hz, 1H, CH), 5.81 (d, *J* = 4 Hz, 1H, thioph), 6.9 (d, *J* = 4 Hz, 1H, thioph), 7.0 (d, *J* = 4 Hz, 1H, thioph), 7.15 (d, *J* = 4 Hz, 1H, thioph), 8.3 (s, 1H, CH=N). CI-MS m/z 381 $(M+1)^+$.

Acosamine Schiff base-ester (21a). A solution of Schiff base hydrochloride (**20a**) (0.62 mg, 0.0014 mmol), the triazole amide (**10a**) (0.88 mg, 0.0029 mmol) and DBU (0.44 mL, 0.0029 mmol) in anhydrous dichloromethane (0.4 mL) was refluxed 4 h and then was stirred overnight at rt. The solvent was evaporated, and the residue was purified by preparative TLC (silica gel, 50% ethyl acetate-hexane, 0.1% triethylamine) to afford the bisderivative (0.5 mg, 50%). ^1H-NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 3H, CH_3), 2.0 (dd, 1H, 2-CH), 2.2 (dt, 1H, 2-CH), 2.96 (s, 6H, $N(CH_3)_2$), 2.99 (s, 6H, $N(CH_3)_2$), 3.43 (s, 3H, OCH_3), 3.77 (m, 1H, 5-CH), 3.98 (m, 1H, 3-CH), 4.85 (d, *J* = 2.4 Hz, 1H, 1-CH), 5.05 (m, 1H, 4-CH), 5.79 (d, *J* = 4 Hz, 1H, thioph), 5.8 (d, *J* = 4 Hz, 1H, thioph), 6.75 (d, *J* = 4 Hz, 1H, thioph), 6.83 (d, *J* = 4 Hz, 1H, thioph), 6.95 (d, *J* = 4, 1H, thioph), 6.98 (d, *J* = 4 Hz, 1H, thioph), 7.02 (d, *J* = 4 Hz, 1H, thioph), 7.58 (d, *J* = 4 Hz, 1H, thioph), 8.3 (s, 1H, CH=N). CI-MS *m/z* 616 $(M+1)^+$

Acosamine amide-ester (22a). A solution of methyl-α-L-acosamidine hydrochloride (**19**) (0.54 mg, 0.0027 mmol), the triazole amide (**10a**) (1.7 mg, 0.0056 mmol) and DBU (0.86 mL, 0.0057 mmol) in anhydrous dichloromethane (0.5 mL) was refluxed for 3 h. The mixture was filtered and the solvent was evaporated under reduced pressure. The residue was purified by preparative TLC (2:1 ethyl acetate-hexane) to afford a yellow solid (1.3 mg, 70% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.35 (d, J = 6.5 Hz, 3H, CH_3), 1.90 (m, 1H, 2-CH), 2.6 (dt, 1H, 2-CH), 2.98 (s, 6H, $N(CH_3)_2$, 2.99 (s, 6H, $N(CH_3)_2$), 3.43 (s, 3H, OCH_3), 4.15 (m, 1H, CH), 4.6 (m, 1H, CH), 4.75 (d, 1H, CH), 4.8 (d, 1H, CH), 5.85 (d, J = 4 Hz, 1H, thioph), 5.95 (s, 1H, NH), 6.52 (d, J = 4 Hz, 1H, thioph), 6.85 (d, 1H, J = 4 Hz, thioph), 6.88 (d, J = 4 Hz, 1H, thioph), 6.98 (d, 1H, J = 4 Hz, thioph), 7.05 (d, J = 4 Hz, 1H, thioph), 7.28 (d, 1H, J = 4 Hz, thioph), 7.67 (d, 1H, J = 4 Hz, thioph). CI-MS m/z 636 $(M+1)^+$. FAB-HRMS for $C_{29}H_{33}N_3O_5S_4$ calcd 631.1303, found 631.1313.

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