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Stereoselective Synthesis of Thiophenedimethyl- and Benzenedimethyl- α,α′-Bridged Bis(glycines)

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Conformationally constrained cystine analogues have been synthesized which have an all-carbon backbone-chain as a bridge between the α,α′-positions in two glycine units. An aromatic ring consisting of a 1,2-disubstituted benzene or a 2,3- and 2,5-disubstituted thiophene has been inserted into the bridge. Chiral auxiliaries were used to effect stereoselective syntheses of the (*S,S*)-bis(amino acids). The latter were further derivatized as Fmoc-derivatives suitable for peptide syntheses. The product 2,3-bis[(2*R*,5*S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]-5-methylthiophene has been subjected to X-ray analysis.

The disulfide functionality of the amino acid cystine is commonly involved in the formation of cyclic peptides and in bridging peptide chains in physiologically essential (oligo)peptides and proteins. The ring or interchain disulfide bridging confers conformational constraints on the peptide or protein structure which may be essential for the spacing of the pharmacophoric region and its interaction with the receptor. When cystine exerts mainly a structural function, isosteric structures may be envisaged to take its place, i.e. the disulfide linkage may be replaced by an ethylene unit, and this may be variously substituted for fine-tuning of conformational constraints. Because of the biological implications which may arise from such interactions, we have initiated a program on the preparation of bridged amino acids as substitutes for cystine in peptides and peptidomimetics.[1–5] In its simplest case, when the $-CH_2S-SCH_2-$ bridge between the two glycine carbons in cystine is replaced by a $-(CH_2)_4-$ bridge, the new bridged amino acid is (*S,S*)-α,α′-diaminosuberic acid. In the work reported herein, we have retained the four atoms in the backbone bridge between the two α-glycine carbons except for the bridge in the bis(amino acid) **19**. Conformational constraints have been introduced into the cystine substitute by the insertion of aromatic structures into the bridge [Fig. 1; (**B**)]. The aromatic structure is *ortho*-substituted by two β-alanine groups. In this way the β-alanine carbons are configurationally locked into a planar *cis*-structure. The absence of the disulfide unit in the bridge between two

Fig. 1.

α-glycines also excludes the cystine–cysteine reductive and oxidative processes.

Several chiral auxiliaries are available for amino acid construction.[6] For stereoselective alkylation procedures we prefer the Schöllkopf 'bislactim ether' chiral auxiliary because of the mild conditions used to effect cleavage of the alkylated product which liberates the desired amino acid ester.

The difunctional alkylating aryl reagents used for the preparation of the bridged amino acids are shown in Scheme 1. Dilithiation of thiophene-2-carboxylic acid with LDA at −78 °C and subsequent alkylation leads to 5-alkylthiophene-2-carboxylic acids. 5-Methylthiophene-2-carboxylic acid was prepared by this procedure.[7] Using butyllithium as the base under similar conditions and quenching the dilithiated species with carbon dioxide is reported to give selectively thiophene-2,3-dicarboxylic acid (**2a**).[8] In our hands the product obtained also contained some of the isomer thiophene-2,5-dicarboxylic acid (**2b**). No attempt was made to separate the regioisomers, and the isomer mixture was reduced directly to the

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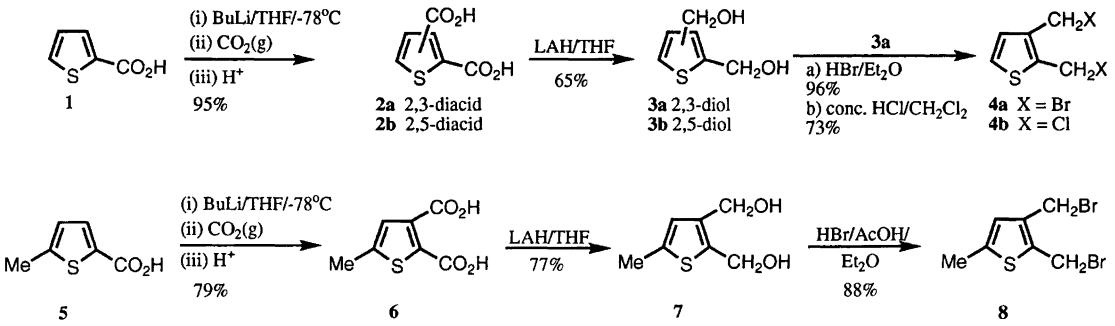


Scheme 1.

corresponding diols **3** by LAH. The isomeric diols were readily separated by chromatography. When the 5-position in thiophene-2-carboxylic acid is occupied by a methyl group (**5**), dilithiation and carboxylation proceed readily to the 2,3-dicarboxylic acid **6** which again was reduced by LAH to the diol **7**. The bromide **4a** was available from the diol **3a** by the reaction with triphenylphosphine–carbon tetrabromide,[8b] or better by treatment of the diol with HBr (g), whereas the dichloride **4b** was prepared from the diol **3a** by the reaction with HCl (aq) in dichloromethane. 40% HBr in acetic acid was used for the preparation of the dibromide **8** from the diol **7**.

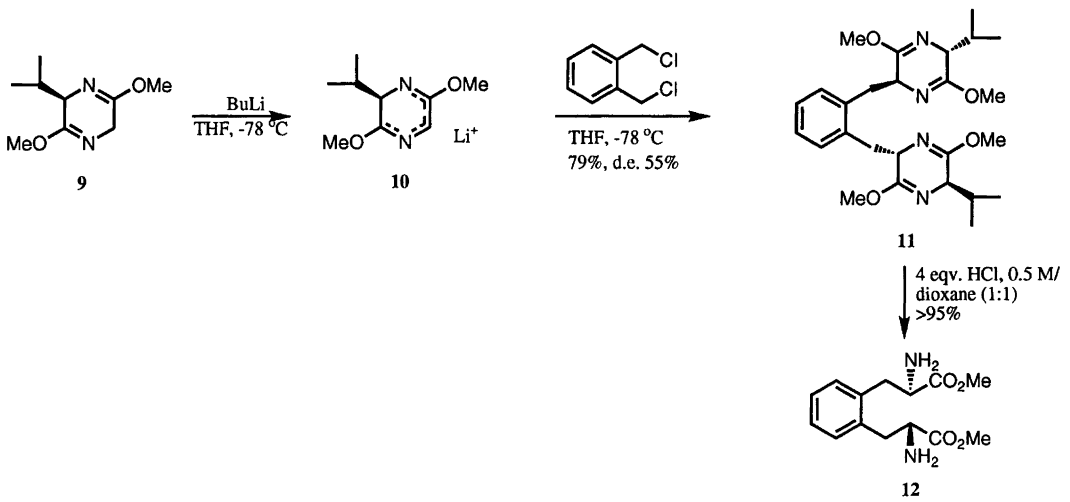
2,5-Bis(chloromethyl)thiophene (**17**) (Scheme 4) was available by chloromethylation of thiophene.[9]

The synthesis of the *o*-xylene-bridged precursor **11** for the bis(amino acid) **12** was effected by alkylation of lithiated (2*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine with α,α′-dibromo-*o*-xylene in THF at −78 °C (Scheme 2). The dialkylated product **11** was isolated in 79% yield, but the stereoselectivity in the overall reaction was low, 55% d.e. The stereoisomers, however, were readily separated; the major isomer was isolated by flash chromatography or by fractional crystallization from acetonitrile.

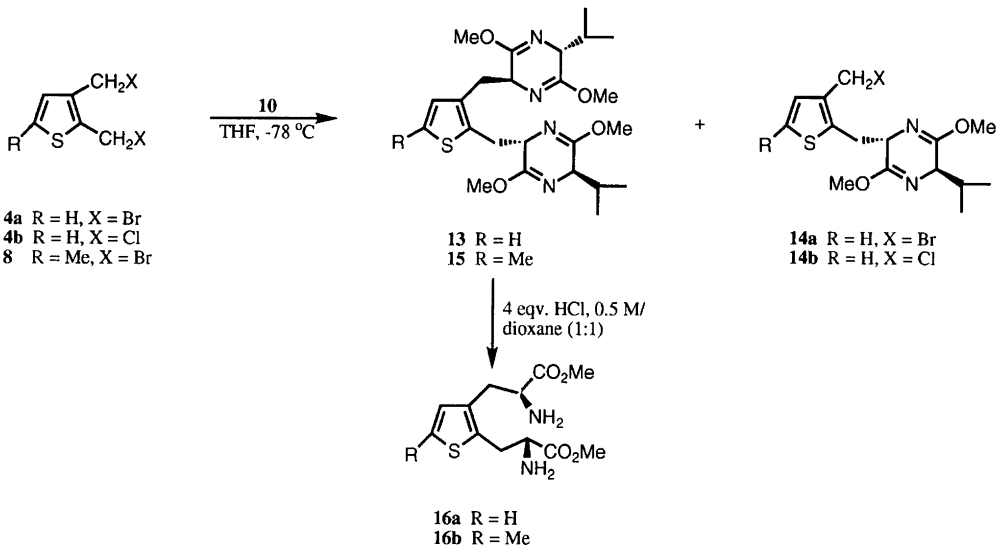
The stereochemical course of this alkylation and of the related alkylations to be discussed below, is readily ascertained by GLC and by NMR (^1H, ^{13}C) because of diastereoisomerism. For the same reason, any significant racemization in the subsequent hydrolytic or acylation reactions (*vide infra*) can be detected by NMR techniques.

Unlike the xylene derivative **11**, the products from the reaction with 2,3-bis(halomethyl)thiophenes are unsymmetrical (Scheme 3). The dialkylated product **15** was formed from the 5-methyl derivative **8** in 65% yield in low stereoisomer ratio (2:1); the isomers were separated by flash chromatography. The structure **15**, which was assigned to the major isomer, has been established by X-ray analysis (*vide infra*, Fig. 2, Tables 1 and 2). The main product from the alkylation with the dibromide **4a** was the dialkylated product **13** (39%, d.e. 43%) together with a significant amount (19%) of monoalkylated product **14a**. The dichloride **4b** gave the same types of product but in somewhat different ratios: dialkylated product **13** (31%, d.e. 49%) and monoalkylated product **14b** (31%). These observations can be rationalized in terms of a competitive metal–proton exchange between the metallated bis-lactim ether and the proton in the thiophene 5-position. It was for this reason that a methyl group was introduced into the thiophene 5-position (**8**) for the alkylation reaction which proceeded satisfactorily to give only the dialkylated product **15** (*vide supra*).

In the 2,5-derivative **19** the alanine substituents are



Scheme 2.



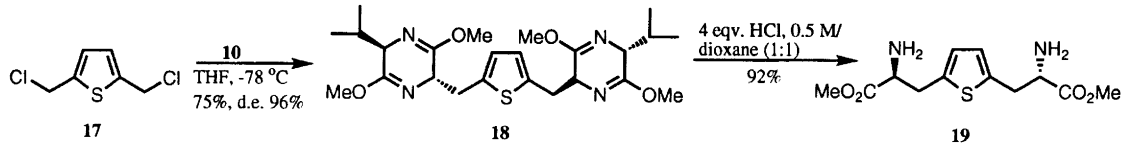
4a R = H, X = Br
4b R = H, X = Cl
8 R = Me, X = Br

13 R = H
15 R = Me

14a R = H, X = Br
14b R = H, X = Cl

16a R = H
16b R = Me

Scheme 3.



17 **18** **19**

Scheme 4.

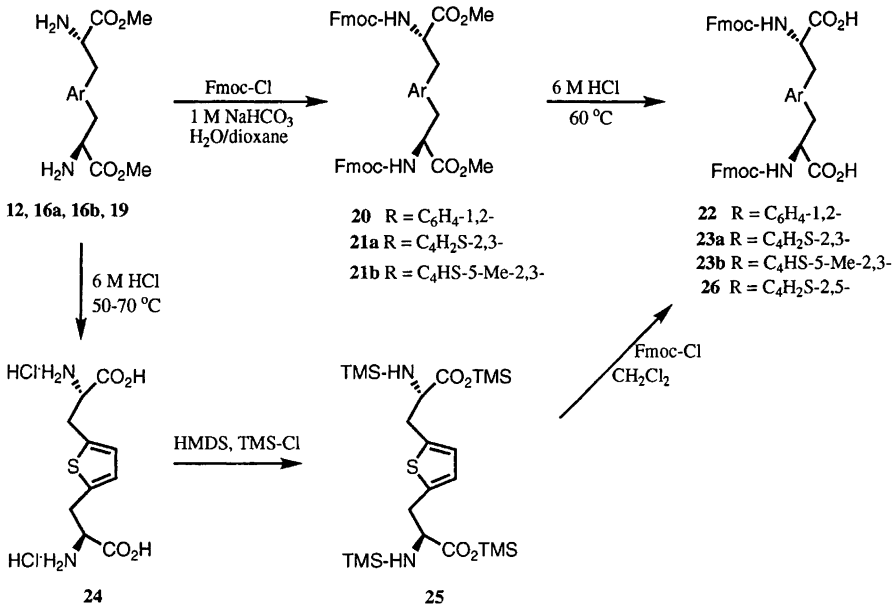
further separated than in the *ortho* isomers; a sulfur atom has been inserted (Scheme 4). Alkylation of the metallated bis-lactim with the dichloride **17** gave the dialkylated product **18** in good yield and high d.e. (96%).

When Schöllkopf's method for acid-catalyzed hydrolysis of the alkylated (2*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazines **11**, **13**, **15** and **18** was strictly adhered to, the yields of the bis(amino acids) as the methyl esters **12**, **16** and **19** were in the range 85–100%. The mild conditions exclude racemization (NMR). Acid hydrolysis of the methyl esters could be effected under strongly acidic conditions (**24**, **25**).

The *ortho* isomer (*S,S*)-α,α'-bis(glycin-2-yl)-*o*-xylene has previously been prepared by vigorous acid-catalyzed cleavage of α,α'-bis[(2*S*,5*S*)-2-*tert*-butyl-1-*tert*-butyloxycarbonyl-3-methyl-4-oxoimidazolidin-5-yl]-*o*-xylene, which itself was prepared by alkylation of lithiated (*S*)-2-*tert*-butyl-1-*tert*-butyloxycarbonyl-3-methyl-4-imidazolidinone using 1,2-bis(bromomethyl)benzene.[10] Attempts to prepare the *ortho* isomer by reactions involving the Heck coupling from appropriately substituted benzenes were not successful although regioisomers were available by this route.[11]

The amino acids have been derivatized further for solid-phase peptide synthesis (Scheme 5). The procedure to be used requires protection of the amino groups as 9-fluorenylmethyloxycarbonyl (Fmoc) derivatives. Amino group protection with 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) was carried out in aqueous dioxane in the presence of sodium bicarbonate as base. This was readily affected taking due care of the fact that the Fmoc-protecting group is readily cleaved by base, especially by secondary amines; piperidine is frequently used for its removal. On the other hand, the Fmoc-protecting group is very resistant to acid cleavage, and therefore the Fmoc-protected esters **20**, **21a** and **21b** can be hydrolyzed to the corresponding Fmoc-protected amino acids **22** and **23** by heating in 6 M HCl. In an alternative procedure for the introduction of the Fmoc-protecting group, the ester **19** was initially hydrolyzed to acid **24** by being heated in 6 M HCl, and the acid **24** treated with an excess of hexamethyldisilazane (HMDS) in the presence of trimethylsilyl chloride; the latter appears to function as a catalyst for the silylation reaction. The product is presumably the persilylated derivative **25**. The silylation was carried out in order to solubilize the amino acid **24** in dichloromethane for the acylation with Fmoc-Cl. Formation of volatile TMS–Cl in the reaction explains why no base was added. The acylated TMS-ester initially formed in this reaction is hydrolyzed to the acid **26** on addition of water. Peptide syntheses involving these functionalized amino acids are to be described elsewhere or have been partly described.[3]

The structure and stereochemistry of the alkylation product from 2,3-bis(bromomethyl)-5-methylthiophene (**8**) has been verified as 2,3-bis[(2*R*,5*S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl) methyl]-5-methylthiophene (**15**) by X-ray analysis. The ORTEP perspective drawing of compound **15** in Fig. 2 clearly shows the *trans*-relationship between the 2-isopropyl groups in the two



Scheme 5.

almost planar pyrazine rings and the 5-substituent containing the thiophene ring. Bond lengths and angles are given in Tables 1 and 2, respectively.

Experimental

Crystallograhic data, experimental conditions and methology for the X-ray analysis of 2,3-bis-[(2*R*,5*S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]-5-methylthiophene (**15**) are given in Table 3. Fractional atomic coordinates are listed in Tables 4 and 5.

The ^{1}H NMR spectra were recorded at 300 MHz with a Varian Unity Plus 300, at 200 MHz with a Varian Gemini 200 instrument, at 200 MHz with a Bruker DPX 200 or at 300 MHz with a Bruker DPX 300. The ^{13}C NMR spectra were recorded at 75 or 50 MHz. Chemical shifts are reported in ppm using $CDCl_3$ (77.00 ppm), DMSO-d_6 (39.50 ppm), DMF-d_7 (70.70 ppm), TMS (0.00 ppm) or 3-trimethylsilyl-1-propanesulfonic acid (0.00 ppm) as references for the ^{13}C spectra, and residual $CHCl_3$ (7.24 ppm), DMSO (2.49 ppm), HDO (4.63 ppm), DMF (2.74 ppm) or TMS (0.00 ppm) as references for the ^{1}H spectra. The mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing potential, and methane or ammonia was used for chemical ionization (CI); the spectra are presented

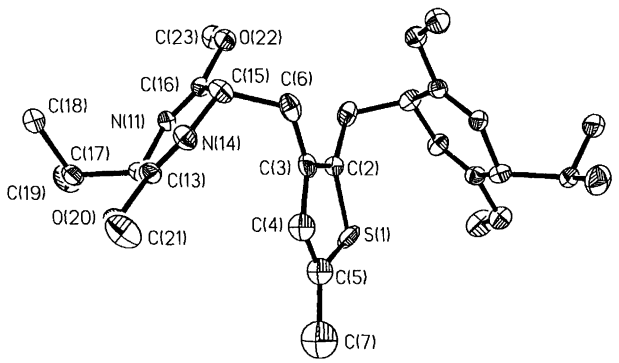


Fig. 2. Perspective ORTEP drawing of 2,3-bis[(2*R*,5*S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]-5-methylthiophene (**15**).

Table 1. Bond lengths (in Å) for compound **15**.

C(2)–C(3)	1.378(8)	N(14)–C(15)	1.461(6)
C(2)–S(1)	1.711(6)	C(15)–C(16)	1.503(6)
C(3)–C(4)	1.469(3)	C(16)–O(22)	1.360(5)
C(3)–C(6)	1.504(6)	C(17)–C(18)	1.520(7)
C(6)–C(15)	1.534(7)	C(17)–C(19)	1.525(6)
N(11)–C(16)	1.263(6)	O(20)–C(21)	1.442(5)
N(11)–C(12)	1.464(6)	O(22)–C(23)	1.444(5)
C(12)–C(13)	1.506(6)	S(1)–C(5)	1.717(8)
C(12)–C(17)	1.530(7)	C(4)–C(5)	1.380(10)
C(13)–N(14)	1.256(6)	C(5)–C(7)	1.506(8)
C(13)–O(20)	1.361(5)		

Table 2. Bond angles (in °) for compound **15**.

C(3)–C(2)–S(1)	108.3(2)	C(16)–C(15)–C(6)	110.6(3)
C(2)–C(3)–C(4)	114.2(5)	N(11)–C(16)–O(22)	121.1(4)
C(2)–C(3)–C(6)	126.2(2)	N(11)–C(16)–C(15)	127.8(4)
C(4)–C(3)–C(6)	119.1(5)	O(22)–C(16)–C(15)	111.1(4)
C(3)–C(6)–C(15)	114.8(4)	C(18)–C(17)–C(19)	110.8(4)
C(16)–N(11)–C(12)	118.2(4)	C(18)–C(17)–C(12)	110.9(4)
N(11)–C(12)–C(13)	112.7(4)	C(19)–C(17)–C(12)	110.9(4)
N(11)–C(12)–C(17)	110.6(4)	C(13)–O(20)–C(21)	116.0(4)
C(13)–C(12)–C(17)	112.5(3)	C(16)–O(22)–C(23)	115.5(3)
N(14)–C(13)–O(20)	120.6(3)	C(2)–S(1)–C(5)	96.1(4)
N(14)–C(13)–C(12)	129.0(4)	C(5)–C(4)–C(3)	111.7(9)
O(20)–C(13)–C(12)	110.4(4)	C(4)–C(5)–C(7)	129.4(8)
C(13)–N(14)–C(15)	117.4(3)	C(4)–C(5)–S(1)	109.3(7)
N(14)–C(15)–C(16)	113.5(4)	C(7)–C(5)–S(1)	121.2(8)
N(14)–C(15)–C(6)	110.2(4)		

Table 3. Crystallographic data for compound **15** and conditions for X-ray analysis.

Empirical formula	$C_{25}H_{38}N_4O_4S$
Formula weight	490.65
Crystal size	0.20 × 0.32 × 0.56 mm
Crystal habit	Parallelepiped
Crystal color	Colorless
Crystal system	Monoclinic
Space group	*C*2
Unit cell dimensions	a=21.300(6) Å
	b=9.674(3) Å β=104.27°
	c=6.986(2) Å
Volume	1395.1(7) Å^3
Z	2
Density (calculated)	1.168 Mg m^{-3}
Absorption coefficient	1.312 mm^{-1}
Absorption correction	XABS2[a] (T_{min}, T_{max}: 0.47, 0.77)
Diffractometer	Siemens P4/RA
Temperature	120(1) K
Radiation source	Rotating anode
Wavelength	1.541 78 Å (Cu Kα)
Monochromator	Ni filter
2θ range for data collection	0–112°
Scan type	θ/2θ
Index ranges	$-16 \leqslant h \leqslant 16$, $-3 \leqslant k \leqslant 10$, $0 \leqslant l \leqslant 7$
Reflections collected	1183
Independent reflections	1157 (R_{int}=0.034)
Observed [$I > 2s(I)$] reflections	1114
Standard reflections	2 every 200 refl.
Percentage decay of standards	Stable to within ±0.8%
Solution and refinement	
System for solution	SHELXTL
Structure solution	Automatic Patterson (SHELXS)
System for refinement	SHELXL-93[13]
Refinement method	Full-matrix least-squares on F^2
Hydrogen atoms	Riding, included in refinement
Extinction correction	None
Data/restraints/parameters	1157/8/159
Final *R* indices[b,c] [$I > 2s(I)$]	$R1$=0.0431, $wR2$=0.1112
Goodness-of-fit[d] on F^2	0.760
Weighting scheme	Calc.
$w = 1/[s^2(F_o^2) + (0.0900P)^2 + 6.9306P]$	
	where $P = (F_o^2 + 2F_c^2)/3$
R indices (all data)	$R1$=0.0465, $wR2$=0.1232
Absolute structure parameter	0.04(6)
Largest diff. peak and hole	0.216 and −0.161 e Å^{-3}

[a]An empirical absorption correction program.[12] [b]$R1 = \Sigma \| F_o - F_c \| / \Sigma | F_o |$. [c]$wR2 = \{\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[w(F_o^2)^2]\}^{1/2}$ [d]Goodness-of-fit = $\{\Sigma[w(F_o^2 - F_c^2)^2] / (M - N)\}^{1/2}$ where M is the number of reflections and N is the number of parameters refined.

Table 4. Fractional atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for compound **15**.

Atom	*x*	*y*	*z*	U_{ea}[a]
C(2)[b]	81(2)	1283(5)	−5896(6)	27(1)
C(3)	−81(2)	1283(5)	−4104(6)	27(1)
C(6)	−161(2)	2536(6)	−2913(6)	27(1)
N(11)	−1723(2)	2316(5)	−5934(5)	21(1)
C(12)	−1910(2)	1120(6)	−4915(6)	21(1)
C(13)	−1572(2)	1085(5)	−2751(6)	22(1)
N(14)	−1123(2)	1844(5)	−1804(5)	25(1)
C(15)	−864(2)	2883(6)	−2920(6)	25(1)
C(16)	−1266(2)	3071(5)	−4997(6)	20(1)
C(17)	−2647(2)	1039(6)	−5277(6)	25(1)
C(18)	−2907(3)	2253(6)	−4321(8)	37(1)
C(19)	−2969(2)	970(7)	−7481(7)	41(1)
O(20)	−1812(2)	44(4)	−1831(4)	28(1)
C(21)	−1517(3)	−127(7)	246(6)	41(2)
O(22)	−1076(2)	4212(4)	−5846(4)	25(1)
C(23)	−1394(2)	4419(6)	−7904(6)	28(1)
S(1)	184(2)	−394(3)	−6532(5)	35(1)
C(4)	−88(11)	−95(6)	−3227(21)	35
C(5)	0(6)	−1126(8)	−4501(12)	35(3)
C(7)	1(7)	−2671(9)	−4235(17)	69(4)

[a]U_{eq} is one-third of the trace of the orthogonalized U_{ij} tensor. [b]C(2) is a symmetry equivalent of C(3).

Table 5. Hydrogen fractional atomic coordinates (× 10^3) and displacement parameters (Å^2 × 10^3) for compound **15**.

Atom	*x*	*y*	*z*	U_{eq}
H(6A)	19(2)	3312(6)	−3450(6)	35
H(6B)	86(2)	2408(6)	−1576(6)	35
H(12A)	−1771(2)	316(6)	−5500(6)	30
H(15A)	−865(2)	3749(6)	−2248(6)	35
H(17A)	−2753(2)	204(6)	−4682(6)	35
H(18A)	−2704(3)	2275(6)	−2932(8)	50
H(18B)	−2812(3)	3093(6)	−4924(8)	80
H(18C)	−3367(3)	2163(6)	−4514(8)	80
H(19A)	−2805(2)	196(7)	−8066(7)	50
H(19B)	−3429(2)	879(7)	−7679(7)	50
H(19C)	−2874(2)	1809(7)	−8088(7)	50
H(21A)	−1720(3)	−884(7)	752(6)	50
H(21B)	−1063(3)	−313(7)	441(6)	50
H(21C)	−1574(3)	706(7)	930(6)	50
H(23A)	−1229(2)	5240(6)	−8378(6)	40
H(23B)	−1314(2)	3637(6)	−8656(6)	40
H(23C)	−1852(2)	4514(6)	−8047(6)	40

as *m/z* (% rel. int.). Fast atom bombardment spectra (FAB-MS) were performed at M-Scan Ltd., Ascot England. Dry THF was distilled from sodium and benzophenone.

Thiophene-2,3-dicarboxylic acid (**2a**) *and thiophene-2,5-dicarboxylic acid* (**2b**). Precooled (−78 °C) BuLi (39 ml, 85.8 mmol, 2.2 M in hexane) was added to a solution of thiophene-2-carboxylic acid (5.00 g, 39.0 mmol) in dry THF (150 ml) at −78 °C. The reaction was stirred for 30 min before being quenched with solid CO_2. The reaction mixture was allowed to reach −10 °C and acidified with 2 M HCl (50 ml) after which the organic layer was separated and the aqueous layer extracted with ethyl acetate (3 × 50 ml). The dried ($MgSO_4$) organic solution was then evaporated and the residue triturated with hexane. The solid residue was the title compounds, ratio **2a**:**2b** 5.5:1; yield 6.36 g (95%). This mixture was reduced without separation of the isomers.

Thiophene-2,3-dicaboxylic acid (**2a**): ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.43 (d, *J* 5.1 Hz, 1 H, H-4), 7.84 (d, *J* 5.1 Hz, 1 H, H-5), 12.61 (br s, 2 H, CO_2H).

Thiophene-2,5-dicarboxylic acid (**2b**):[14] ^{1}H NMR

(200 MHz, DMSO-d_6): δ 7.73 (s, 2 H, H-3 and H-4), 12.61 (br s, 2 H, CO_2H).

2,3-Bis(hydroxymethyl)thiophene (**3a**) *and 2,5-bis-(hydroxymethyl)thiophene* (**3b**). The above mixture of thiophene-2,3-dicarboxylic acid and thiophene-2,5-dicarboxylic acid (2.00 g, 11.6 mmol) was added to a slurry of LAH (2.20 g, 58.0 mmol) in dry THF (60 ml), under nitrogen at 5 °C. The reaction mixture was stirred at ambient temperature for 6 h, cooled to 5 °C, quenched by addition of saturated ammonium chloride solution (60 ml), and the aluminium precipitates were dissolved by addition of 2 M HCl (80 ml). The mixture was filtered through glass wool and the aqueous layer was extracted with dichloromethane (3 × 100 ml). The combined extracts were dried ($MgSO_4$), and evaporated, and the 2,3- and 2,5-isomers were separated by flash chromatography using dichloromethane and 5% methanol in dichloromethane.

2,3-Bis(hydroxymethyl)thiophene (**3a**): 0.94 g (57%); colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.70 (br s, 2 H, 2 × OH), 4.64 (s, 2 H, CH_2), 4.74 (s, 2 H, CH_2), 6.99 (d, *J* 5.1 Hz, 1 H, H-4), 7.16 (d, *J* 5.1 Hz, 1 H, H-5).

2,5-Bis(hydroxymethyl)thiophene (**3b**):[15] 0.13 g (8%); colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.23 (br s, 2 H, 2 × OH), 4.74 (s, 4 H, 2 × CH_2), 6.83 (s, 2 H, H-3 and H-4).

2,3-Bis(bromomethyl)thiophene (**4a**). HBr (g) was bubbled through a solution of 2,3-bis(hydroxymethyl)-thiophene (1.04 g, 7.21 mmol) in diethyl ether (250 ml) at 0 °C for 1 h and the solution was stirred at ambient temperature overnight. After this time, water (100 ml) was added, the ether layer was separated and the aqueous layer was extracted with diethyl ether (50 ml). The combined ether phases were shaken with saturated sodium bicarbonate solution (2 × 20 ml), dried ($MgSO_4$) and the ether evaporated off; yield 1.86 g (96%); light brown oil which crystallized on storage in the cold but gradually polymerized. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.51 (s, 2 H, CH_2), 4.72 (s, 2 H, CH_2), 6.99 (d, *J* 5.2 Hz, 1 H, H-4), 7.23 (d, *J* 5.2 Hz, 1 H, H-5).

2,3-Bis(chloromethyl)thiophene (**4b**). A mixture of 2,3-bis(hydroxymethyl)thiophene (177 mg, 1.23 mmol) and conc. HCl (5 ml) in dichloromethane (20 ml) was stirred vigorously at ambient temperature for 2 h, after which the phases were separated and the aqueous layer extracted with dichloromethane (3 × 10 ml). The combined organic extracts were washed with saturated sodium bicarbonate solution, dried ($MgSO_4$) and evaporated, and the product was purified by flash chromatography using hexane–ethyl acetate 9:1; yield 162 mg (73%); light yellow oil. Found C, 40.21; H, 3.82. Calc. for $C_6H_6Cl_2S$: C, 39.80; H, 3.34. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.64 (s, 2 H, CH_2), 4.83 (s, 2 H, CH_2), 7.05 (d, *J* 5.1 Hz, 1 H, H-4), 7.27 (d, *J* 5.1 Hz, 1 H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 37.85 (CH_2), 38.17 (CH_2), 125.29 (C-5), 128.46 (C-4), 135.89, 136.33 (C-2 and C-3). MS(EI): 182/180 (17/25, M^+), 147 (36), 145 (100), 110 (74), 109 (23).

5-Methylthiophene-2,3-dicarboxylic acid (**6**). BuLi (24 ml, 52.80 mmol, 2.2 M in hexane) was added dropwise to a solution of 5-methylthiophene-2-carboxylic acid[7] (3.21 g, 22.58 mmol) in dry THF (100 ml) under nitrogen at −78 °C and the reaction mixture was stirred at this temperature for 30 min. CO_2 (g) was bubbled through the solution for 30 min and the reaction mixture allowed to reach −10 °C before 2 M HCl (50 ml) was added. The two layers were separated, the aqueous phase extracted with ethyl acetate (3 × 50 ml), the combined organic phases were dried ($MgSO_4$) and evaporated and the residue was triturated with hexane (3 × 15 ml). The product was a pale yellow solid, m.p. 210–212 °C (Et_2O); yield 3.33 g (79%). Anal. $C_7H_6O_4S$: C, H. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.47 (d, *J* 1 Hz, 3 H, CH_3), 7.16 (d, *J* 1 Hz, 1 H, H-4). ^{13}C NMR (50 MHz, DMSO-d_6): δ 11.31 (CH_3), 124.69 (C-4), 129.40, 133.43, 141.37 (C-2, C-3 and C-5), 158.57 (C=O), 161.46 (C=O). MS(EI): 186 (16, M^+), 142 (100), 125 (50), 124 (13), 97 (77), 96 (17), 69 (11), 53 (16), 45 (21), 44 (41).

2,3-Bis(hydroxymethyl)-5-methylthiophene (**7**). A solution of 5-methylthiophene-2,3-dicarboxylic acid (0.74 g, 3.96 mmol) in THF (5 ml) was added dropwise to a slurry of LAH (0.76 g, 20.00 mmol) in THF (20 ml) under nitrogen at −20 °C. The mixture was then stirred at ambient temperature for 6 h, cooled to −20 °C and saturated aqueous NH_4Cl (5 ml) was added carefully. The resultant mixture was filtered, the solid washed with dichloromethane (100 ml) and the filtrate dried ($MgSO_4$) and evaporated. The crude product was a colourless oil; yield 479 mg (77%). The product was used in the subsequent reaction without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.44 (s, 3 H, CH_3), 4.60 (s, 2 H, CH_2), 4.70 (s, 2 H, CH_2), 6.67 (s, 1 H, H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.92 (CH_3), 57.53 (CH_2), 58.67 (CH_2), 127.51 (C-4), 137.72, 138.90, 139.41 (C-2, C-3 and C-5).

2,3-Bis(bromomethyl)-5-methylthiophene (**8**). 2,3-Bis-(hydroxymethyl)-5-methylthiopene (618 mg, 3.90 mmol) was added to a mixture of diethyl ether (20 ml) and HBr in acetic acid (20 ml, 33%) and the mixture was left overnight at ambient temperature. Dichloromethane (30 ml) and water (20 ml) were added and the organic phase was washed with saturated aqueous $NaHCO_3$ (3 × 20 ml), dried ($MgSO_4$) and evaporated; yield 756 mg (88%). The crude product was used in the subsequent reaction without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.42 (d, *J* 1 Hz, 3 H, CH_3), 4.45 (s, 2 H, CH_2), 4.70 (s, 2 H, CH_2), 6.67 (d, *J* 1 Hz, 1 H, H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.93 (CH_3), 24.68 (CH_2), 24.81 (CH_2), 128.04 (C-4), 135.47, 137.35, 141.42

(C-2, C-3 and C-5). MS(EI): 286/284/282 (3/7/3, M^+), 205 (49), 203 (47), 125 (14), 124 (100), 123 (19), 97 (14).

*α,α′-Bis[(2*R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-o-xylene* (**11**). BuLi (13 ml, 19.50 mmol, 15 M in hexane) was added dropwise to a solution of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (3.50 g, 19.00 mmol) in dry THF (8 ml) under nitrogen at −78 °C. The mixture was stirred for 10 min before the dropwise addition of a solution of α,α′-dichloro-*o*-xylene (1.66 g, 9.50 mmol) in THF (5 ml). The reaction mixture was allowed to warm to ambient temperature overnight, the solvent was evaporated off and the crude product was purified by flash chromatography using hexane–ethyl acetate 4:1; yield 3.51 g (79%), d.e. 55%. The main diastereomer was isolated by flash chromatography or by fractional recrystallization from acetonitrile; pale yellow crystalline material, m.p.122–124 °C (MeCN). Anal. $C_{26}H_{38}N_4O_4$: C, H. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.59 (d, *J* 6.7 Hz, 6 H, 2 × CH_3), 0.94 (d, *J* 6.8 Hz, 6 H, 2 × CH_3), 2.13 (m, 2 H, 2 × CH), 2.94, 3.45 (ABX, 4 H, 2 x CH_2), 3.28 (dd, 2 H, 2 × H-2), 3.62 (s, 6 H, CH_3O), 3.71 (s, 6 H, CH_3O), 4.26 (dd, 2 H, H-5), 7.05 (m, 4 H, 4 x CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.38 (2 × CH_3), 18.98 (2 × CH_3), 30.96 (2 × CH), 36.49 (2 × CH_2), 52.10 (2 × CH_3O), 52.20 (2 × CH_3O), 57.14 (2 × C-5), 59.98 (2 × C-2), 125.86 (2 × CH), 130.41 (2 × CH), 136.75 (2 × C), 162.85 (2 × C), 163.61 (2 × C). MS(CI–CH_4): 472 (30, $M+1$), 471 (100), 469 (18), 439 (21), 428 (10), 427 (37), 287 (11), 183 (13), 141 (11), 57 (10).

*Dimethyl (*S,S*)-α,α′-diamino-1,2-benzenedipropionate* (**12**). α,α′-Bis[(2*R*,5*S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-*o*-xylene (1.06 g, 2.25 mmol) was added to 0.5 M HCl (18.0 ml, 9.00 mmol) in dioxane (18 ml) and the mixture was stirred at ambient temperature for 6 h. The solution made basic by addition of conc. NH_3 (pH 10), extracted with dichloromethane (3 × 30 ml) and the combined organic layers were dried ($MgSO_4$) and evaporated. The valine methyl ester was removed by slow bulb-to-bulb distillation at 50 °C/0.3 mmHg. The residual product was a yellowish oil; 0.63 g (>95%). Anal. $C_{14}H_{18}N_2O_4$: C, H. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.63 (s, 4 H, NH_2), 3.02 (m, 4 H, 2 × CH_2), 3.71 (s, 6 H, 2 × CH_3O), 3.74 (m, 2 H, 2 × CH), 7.18 (m, 4 H, 4 × CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.26 (2 × CH_2), 52.47, 56.10 (2 × CH and 2 × CH_3O), 127.48 (2 × CH) 130.80 (2 × CH), 136.73 (2 × C), 175.92 (2 × C=O). MS(CI–CH_4) 281 (100, $M^+ + 1$), 204 (42), 88 (12).

*2,3-Bis[(2*R,5S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]thiophene* (**13**) *and 3-bromomethyl-2-[(2*R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]methylthiophene* (**14a**) BuLi (1.50 ml, 3.00 mmol, 2.2 M in hexane) was added dropwise to a solution of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (581 mg, 3.16 mmol) in THF (10 ml) under nitrogen at −78 °C. The mixture was stirred for 10 min before the dropwise addition of a solution of 2,3-bis-(bromomethyl)thiophene (426 mg, 1.58 mmol) in THF (4 ml). The addition was complete within 10 min. The reaction mixture was allowed to warm to ambient temperature overnight, 0.1 M phosphate buffer solution pH 7 (10 ml) added and the aqueous layer was extracted with dichloromethane (3 × 20 ml). The combined organic layers were dried ($MgSO_4$) and concentrated and the products were separated by flash chromatography using hexane–ethyl acetate 9:1. 3-Bromomethyl-2-[(2*R*, 5*S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-methylthiophene (**14a**) was first eluted; yield 114 mg (19%), colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.64 (d, *J* 6.8 Hz, 3 H, CH_3), 0.85 (d, *J* 6.8 Hz, 3 H, CH_3), 2.19 (m, 1 H, CH), 3.34 (dd, 2 H, CH_2), 3.63 (dd, 1 H, H-2′), 3.69 (s, 3 H, CH_3O), 3.71 (s, 3 H, CH_3O), 4.28 (dd, 1 H, H-5′), 4.48–4.57 (AB, *J* 10.5 Hz, 2 H, CH_2Br), 6.94 (d, *J* 5.2 Hz, 1 H, H-4), 7.07 (d, *J* 5.2 Hz, 1 H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.59 (CH_3), 18.96 (CH_3), 26.18 (CH_2Br), 31.66 (CH), 31.98 (CH_2), 52.34 (CH_3O), 52.59 (CH_3O), 55.77 (C-5′), 60.86 (C-2′), 124.25 (C-4), 128.23 (C-5), 135.03, 137.35 (C-2 and C-3), 161.77 (C), 164.71 (C). MS(EI): 374/372 (2/2, M^+), 331/329 (5/5), 293 (12), 278 (8), 235 (8), 183 (55), 141 (100), 110 (40). HR-MS(EI): 374.0529/372.0536. Calc. for $C_{15}H_{21}BrN_2O_2S$: 374.048665/372.050711. The regiochemistry of the monoalkylation was confirmed by long-range HETCOR, which showed a correlation between CH_2Br and C4.

*2,3-Bis[(2*R,5S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]thiophene* (**13**). Yield 290 mg (39%), white solid material (pure by GLC and NMR), d.e. 43%. The stereoisomers were not separated. M.p. 124 –126 °C. Anal. $C_{24}H_{36}N_4O_4S$: C, H. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.57 (d, *J* 6.8 Hz, 3 H, CH_3), 0.61 (d, *J* 6.8 Hz, 3 H, CH_3), 0.92 (d, *J* 6.8 Hz, 3 H, CH_3), 0.94 (d, *J* 6.8 Hz, 3 H, CH_3), 2.09–2.20 (m, 2 H, 2 × CH), 2.97–3.18 (m, 2 H, CH_2), 3.25–3.30 (m, 2 H, CH_2), 3.27 (m, 1 H, H-2′), 3.53 (dd, 1 H, H-2′), 3.64 (s, 6 H, 2 × CH_3O), 3.66 (s, 3 H, CH_3O), 3.70 (s, 3 H, CH_3O), 4.22 (dd, 2 H, 2 × H-5′), 6.63 (d, *J* 5.2 Hz, 1 H, H-4), 6.92 (d, *J* 5.2 Hz, 1 H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.35 (CH_3), 16.50 (CH_3), 18.94 (CH_3), 18.99 (CH_3), 30.94 (CH), 31.37 (CH), 31.88 (CH_2), 31.92 (CH_2) 52.07 (CH_3O), 52.08 (CH_3O), 52.24 (CH_3O), 52.48 (CH_3O), 56.26 (C-5′), 56.48 (C-5′), 60.08 (C-2′), 60.62 (C-2′), 122.32 (C-5), 128.87 (C-4), 134.55, 134.92 (C-2 and C-3), 162.08 (C), 162.55 (C), 163.94 (C), 164.34 (C). MS(EI): 476 (6, M^+), 434 (17), 433 (66), 294 (19), 293 (96), 141 (100), 111 (11), 110 (25). HR-MS(EI): 476.243078. Calc. for $C_{24}H_{36}N_4O_4S$: 476.245728.

*2,3-Bis[(2*R,5S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]thiophene* (**13**) *and 3-chloromethyl-*

2-[(2R,5S)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]methylthiophene (**14b**). BuLi (0.85 ml, 1.87 mmol, 2.2 M in hexane) was added dropwise to a solution of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (320 mg, 1.74 mmol) in THF (3 ml) under nitrogen at −78 °C and the mixture was stirred for 10 min before the dropwise addition of a solution of 2,3-bis(chloromethyl)thiophene (157 mg, 0.87 mmol) in THF (2 ml). The addition was complete within 10 min. The reaction mixture was allowed to warm to ambient temperature overnight, 0.1 M phosphate buffer pH 7 (5 ml) was added, the aqueous layer extracted with dichloromethane (3 × 10 ml), the combined organic layers dried ($MgSO_4$), concentrated, and the products separated by flash chromatography using hexane–ethyl acetate 9:1.

3-Chloromethyl-2-[(2R,5S)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]thiophene (**14b**) was eluted first; yield 89 mg (31%), colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.63 (d, *J* 6.8 Hz, 3 H, CH_3), 0.96 (d, *J* 6.8 Hz, 3 H, CH_3), 2.18 (m, 1 H, CH), 3.34 (dd, 2 H, CH_2), 3.62 (dd, 1 H, H-2′), 3.68 (s, 3 H, CH_3O), 3.71 (s, 3 H, CH_3O), 4.27 (dd, 1 H, H-5), 4.55–4.64 (AB, *J* 11.7 Hz, 2 H, CH_2Cl), 6.95 (d, *J* 5.2 Hz, 1 H, H-4), 7.07 (d, *J* 5.2 Hz, 1 H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.55 (CH_3), 18.92 (CH_3), 31.60 (CH), 31.84 (CH_2), 38.92 (CH_2Cl), 52.29 (CH_3O), 52.53 (CH_3O), 55.91 (C-5′), 60.80 (C-2′), 124.24 (C-5), 127.92 (C-4), 135.02, 137.01 (C-2 and C-3), 161.70 (C), 164.67 (C). MS(EI): 330/328 (3/1, M^+), 285 (5), 234 (10), 183 (42), 141 (100), 111 (18), 110 (30). HR-MS(EI): 330.0987/328.1030. Calc. for $C_{15}H_{21}ClN_2O_2S$: 330.0983/328.1012. The regiochemistry assigned to the monoalkylated product was confirmed by long range HETCOR, which showed correlation between CH_2Cl and C-4.

2,3-Bis[(2R,5S)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinylmethyl]thiophene (**13**): Yield 133 mg (32%), white solid material (pure by GLC–NMR).

2,3-Bis[(2R,5S)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]-5-methylthiophene (**15**). BuLi (8.7 ml, 20.00 mmol, 2.3 M in hexane) was added dropwise to a solution of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (3.52 g, 19.08 mmol) in dry THF (30 ml) under nitrogen at −78 °C. The mixture was stirred for 30 min, after which a solution of 2,3-bis(bromomethyl)-5-methylthiophene (2.71 g, 9.54 mmol) in THF (5 ml) was added dropwise over 30 min. Stirring was continued for 2 h and thereafter for 24 h at ambient temperature before the addition of water (10 ml). The resultant mixture was extracted with diethyl ether (3 × 20 ml) and the organic solution was dried ($MgSO_4$) and evaporated. GLC of the residual material showed that the two diastereoisomers were formed in the ratio 2:1. The isomers were separated by flash chromatography, hexane–ethyl acetate 9:1; yield of the title product (the main isomer) 3.06 g (65%), m.p. 73 °C. Anal. $C_{25}H_{38}N_4O_4S$: C, H. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.57 (d, *J* 7 Hz, 3 H, CH_3), 0.61 (d, *J* 7 Hz, 3 H, CH_3), 0.94 (d, *J* 6 Hz, 6 H, 2 × CH_3), 2.18 (m, 2 H, CH), 2.26 (s, 3 H, CH_3), 2.85–3.45 (m, 4 H, 2 × CH_2), 3.61 (m, 14 H, 4 × OMe, 2 × CH), 4.18 (m, 2 H, 2 × H-5′), 6.30 (s, 1 H, H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.12 (CH_3), 16.38 (CH_3), 16.50 (CH_3), 18.96 (CH_3), 19.00 (CH_3), 31.03 (CH), 31.42 (CH), 32.01 (CH_2), 32.10 (CH_2), 52.02 (CH_3O), 52.08 (CH_3O), 52.22 (CH_3O), 52.47 (CH_3O), 56.31 (C-5′), 56.50 (C-5′), 60.16 (C-2′), 60.66 (C-2′), 127.17 (C-4), 132.56, 134.43, 136.00 (C-2, C-3, C-5), 162.26 (C), 162.74 (C), 163.81 (C), 164.17 (C). MS(EI): 491 (1.2, M^+), 447 (20), 308 (19), 307 (90), 184 (29), 183 (19), 141 (100), 126 (12) 125 (36), 124 (28), 43 (12).

Dimethyl (S,S)-α,α′-diamino-2,3-thiophenedipropionate (**16a**). Bis([2*R*,5*S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]thiophene (242 mg, 0.51 mmol) was added to 0.5 M HCl (4.46 ml, 2.23 mmol) and dioxane (5 ml), the mixture stirred overnight at ambient temperature before the solution was made alkaline (pH 10) by addition of conc. aqueous ammonia. The aqueous layer was extracted with dichloromethane (2 × 15 ml), and the combined layers were dried ($MgSO_4$) and concentrated and the valine methyl ester removed by slow bulb-to-bulb distillation, at 50 °C, 0.5 mmHg. The residual pale yellow oil, 145 mg (>95%) was the title compound. Anal. $C_{12}H_{18}N_2O_4S$. C, H ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (br s, 4 H, 2 × NH_2), 2.71–3.24 (m, 4 H, 2 × CH_2), 3.51–3.71 (m, 2 H, 2 × CH), 3.64 (s, 3 H, CH_3O), 3.66 (s, 3 H, CH_3O), 6.76 (d, *J* 5.2 Hz, 1 H, H-4), 7.03 (d, *J* 5.2 Hz, 1 H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 32.82 (CH_2), 33.21 (CH_2), 51.82 (CH_3O), 51.93 (CH_3O), 55.11 (CH), 55.74 (CH), 123.79 (C-5), 128.47 (C-4), 134.71, 135.19 (C-2 and C-3), 174.75 (C=O), 175.26 (C=O). MS(EI): 210 (17), 198 (11), 182 (11), 150 (16), 112 (44), 111 (68), 110 (10), 97 (11), 88 (100). HR-MS(EI): 286.0987. Calc. for $C_{12}H_{18}N_2O_4S$: 286.098729.

Dimethyl (S,S)-α,α′-diamino-5-methyl-2,3-thiophenedipropionate (**16b**). 2,3-Bis[2*R*,5*S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]-5-methylthiophene (1.48 g, 3.02 mmol) was added to 0.5 M HCl (27 ml, 13.50 mmol) and dioxane (25 ml) and the mixture stirred at ambient temperature for 5 h. The pH was then adjusted to 10 with conc. aqueous NH_3, the mixture was extracted with dichloromethane (3 × 50 ml), the dried ($MgSO_4$) organic solution was evaporated and the valine methyl ester removed by slow (3 h) bulb to bulb distillation at 50 °C/0.5 mmHg. The residual slightly yellow oil was the title compound; yield 824 mg (91%). Anal. $C_{13}H_{20}N_2O_4S$: C, H. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.64 (br s, 4 H, 2 × NH_2), 2.35 (s, 3 H, CH_3), 2.74–3.14 (m, 4 H, 2 × CH_2), 3.69 (s, 3 H, CH_3O), 3.71 (s, 3 H, CH_3O), 3.65 (m, 2 H, 2 × CH), 6.44 (s, 1 H, s, H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.16 (CH_3), 32.72

(CH_2), 33.20 (CH_2) 51.97, 52.07, 55.10, 55.72 (2 × CH and 2 × CH_3O), 126.59 (C-4), 132.72, 134.54, 137.65 (C-2, C-3 and C-5), 174.97 (C=O), 175.43 (C=O). MS (CI–CH_4): 302 (17), 301 (100), 284 (12), 224 (30), 214 (19), 213 (13), 212 (16), 210 (11), 198 (10), 197 (24), 125 (17), 88 (17).

2,5-Bis[(2R,5S)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)-methyl]thiophene (**18**). BuLi (19 ml, 30.4 mmol, 1.5 M in hexane) was added dropwise to a solution of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (5.00 g, 27.14 mmol) in dry THF (15 ml) under nitrogen at −78 °C. The mixture was stirred for 10 min and 2,5-bis(chloromethyl)thiophene[8] (2.50 g, 13.80 mmol) in THF (10 ml) was added dropwise. The reaction mixture was stirred at ambient temperature overnight, the solvent evaporated off and the crude product purified by flash chromatography using hexane–ethyl acetate 4:1; yield: 4.87 g (75%), white crystalline material. The product contained 2.3% of the (*S,R*)-isomer which was removed by flash chromatography or recrystallization from MeCN; m.p. 79 °C (MeCN). Anal. $C_{24}H_{36}N_4O_4S$: C, H. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.63 (d, *J* 6.9 Hz, 6 H, 2 × CH_3), 0.96 (d, *J* 6.9 Hz, 6 H, 2 × CH_3), 2.16 (m, 2 H, 2 × CH), 3.58 (dd, 2 H, 2 × H-2′), 3.65 (s, 6 H, 2 × CH_3O), 3.71 (s, 6 H, 2 × CH_3O), 4.23 (dd, 2 H, 2 × H-5′), 6.48 (s, 2 H, H-3 and H-4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.56 (2 × CH_3), 18.85 (2 × CH_3), 31.46, 34.59 (2 × CH and 2 × CH_2), 51.94 (2 × CH_3O), 52.41 (2 × CH_3O), 56.04 (2 × C-5′), 60.48 (2 × C-2′), 125.80 (C-3 and C-4), 137.65 (C-2 and C-5), 161.57 (2 × C), 164.43 (2 × C). MS(CI-NH_3): 477 (100, *M*+1), 367 (14), 185 (32), 183 (57).

Dimethyl (S,S)-α,α′-diamino-2,5-thiophenedipropionate (**19**). 2,5-Bis[(2*R*,5*S*)-(2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl)methyl]thiophene (1.01 g, 2.11 mmol) was added to a solution of dioxane (20 ml), HCl (0.7 ml, 8.5 mmol, 12 M) and water (20 ml) and the mixture was stirred at ambient temperature for 6 h, after which time it was extracted with diethyl ether (30 ml). The water phase was collected, made alkaline with conc. aqueous ammonia (pH 10) and extracted with chloroform (3 × 30 ml), and the chloroform extracts combined and dried ($MgSO_4$). The solvents were evaporated off and the valine methyl ester removed by slow bulb to bulb distillation at 50° C/0.5 mmHg; yield 0.56 g (92%), yellowish oil. Anal. $C_{12}H_{16}N_2O_4S$: C, H. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (br s, 4 H, 2 × NH_2), 3.16 (m, 4 H, 2 × CH_2), 3.70 (m, 2 H, 2 × CH), 3.73 (s, 6 H, 2 × CH_3O), 6.68 (s, 2 H, H-3 and H-4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.26 (2 × CH_2), 52.02, 55.52 (2 × CH and 2 × CH_3O), 126.32 (C-3 and C-4), 138.19 (C-2 and C-5), 174.85 (2 × C=O). MS(CI-NH_3): 287 (100, *M*+1), 285 (30), 227 (9), 225 (8), 200 (13).

Dimethyl (S,S)-α,α′-bis(9-fluorenylmethoxycarbonylamino)-1,2-benzenedipropionate (**20**). A mixture of dimethyl (*S,S*)-α,α′-diamino-1,2-benzenedipropionate (294 mg, 1.05 mmol), 9-fluorenylmethyl chloroformate (813 mg, 3.10 mmol) and 1 M $NaHCO_3$ (3.1 ml, 3.10 mmol) in dioxane (5 ml) and water (5 ml) was stirred for 2 h at ambient temperature, after which the mixture was extracted with dicloromethane (3 × 70 ml) and the dichloromethane solution was dried ($MgSO_4$) and evaporated. The residual material was purified by flash chromatography using dichloromethane and 2% methanol in dichloromethane; yield 596 mg (78%) of a white solid m.p. 214 °C (toluene–hexane). Anal. $C_{44}H_{40}N_2O_8$: C, H. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.08–3.25 (m, 4 H, 2 × CH_2), 3.72 (m, 6 H, 2 × CH_3O), 4.09–4.37 (m, 6 H, 4 × CH, 2 × NH), 4.70, 5.51 (m, 4 H, 2 × CH_2), 7.11–7.76 (20 H, m 20 × CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 35.06, 46.97, 52.47, 54.22, 67.11, 119.93, 125.02, 126.97, 127.35, 127.63, 130.18, 134.64, 141.22, 143.71, 155.74, 172.30.

Dimethyl (S,S)-α,α′-bis(9-fluorenylmethoxycarbonylamino)-2,3-thiophenedipropionate (**21a**). A mixture of dimethyl (*S,S*)-α,α′-diamino-2,3-thiophenedipropionate (142 mg, 0.50 mmol), 9-fluorenylmethyl chloroformate (385 mg, 1.50 mmol) and 1 M $NaHCO_3$ (1.5 ml, 1.50 mmol) in dioxane (2 ml) and water (2 ml) was stirred at ambient temperature for 1.5 h. The mixture was then extracted with dichloromethane, the dried ($MgSO_4$) extracts were evaporated and the product was purified by flash chromatography using dichloromethane and dichloromethane–diethyl ether 9:1; yield 234 mg (65%), white solid m.p. 205 °C. Found: C, 70.21; H 5.46. Calc. for $C_{42}H_{38}N_2O_8S$: C, 69.34; H, 5.41. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.11–4.71 (m, 18 H, 4 × CH_2, 4 × CH, 2 × CH_3O), 5.50 (m, 2 H, 2 × NH), 6.81 (d, *J* 5 Hz, 1 H, H-4), 7.17 (d, *J* 5 Hz, 1 H, H-5), 7.32–7.81 (m, 16 H, 16 × CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.22, 30.58, 47.06, 52.53, 52.66, 54.09, 54.60, 67.09, 67.24, 119.96, 124.19, 125.06, 127.04, 127.69, 128.48, 133.72, 141.27, 143.74, 155.64, 171.40, 172.01.

Dimethyl (S,S)-α,α′-bis(9-fluorenylmethoxycarbonylamino)-5-methyl-2,3-thiophenedipropionate (**21b**). A mixture of dimethyl (*S,S*)-α,α′-diamino-5-methyl-2,3-thiophenedipropionate (0.50 g, 1.66 mmol), 9-fluorenylmethyl chloroformate (1.29 g, 5.00 mmol) and 1 M $NaHCO_3$ (5.0 ml, 5.00 mmol) in dioxane (10 ml) and water (10 ml) was stirred at ambient temperature for 45 min. The mixture was then extracted with dichloromethane, the dried ($MgSO_4$) extracts were evaporated and the product was purified by flash chromatography using dichloromethane and 2% methanol in dichloromethane; yield 0.98 g (79%), white solid m.p. 128–130 °C. Found: C, 68.12; H, 5.46. Calc. for $C_{43}H_{40}N_2O_8S$: C, 69.34; H, 5.41. MS(FAB): 745 (30, M^++1), 523 (90), 327 (100). ^{1}H NMR (200 MHz, DMF-d_7): δ 2.33 (s, 3 H, CH_3), 2.72–3.34 (m, 6 H, 2 × CH_2, 2 × CH), 3.68 (s, 3 H, CH_3O), 3.71 (s, 3 H, CH_3O), 4.27 (d, *J* 6 Hz, 4 H, 2 × CH_2), 4.25–4.47 (m, 4 H, 2 × CH, 2 × NH), 6.69

(s, 1 H, H-4), 7.30–8.03 (m, 16 H, 16 × CH). ^{13}C NMR (50 MHz, DMF-d_7): δ 15.10, 47.54, 47.64, 52.37, 52.49, 55.74, 56.44, 67.13, 120.73, 126.09, 127.77, 128.37, 133.52, 135.32, 137.36, 141.79, 144.80, 156.96, 172.66, 173.16.

(S,S)-α,α'-Bis(9-fluorenylmethoxycarbonylamino)-1,2-benzenedipropionic acid (**22**). Dimethyl (*S,S*)-α,α'-bis(9-fluorenylmethoxycarbonylamino)-1, 2-benzenedipropionate (173 mg, 0.24 mmol) was heated with stirring in 6 M HCl (15 ml) and dioxane (15 ml) at 85 °C overnight. The solvents were evaporated off, and the residue was triturated with water (3 × 15 ml), dried and triturated with hexane (3 × 15 ml) which left a white solid; 120 mg (72%), m.p. 248–252 °C. Found: C, 71.51; H, 5.61. Calc. for $C_{42}H_{36}N_2O_8$: C, 72.40; H, 5.21. ^{1}H NMR (200 MHz, DMF-d_7): δ 3.05–4.62 (m, 14 H, 4 × CH_2, 2 × NH, 4 × CH), 7.10–8.05 (m, 20 H, 20 × CH), 13.20 (s, 2 H, 2 × OH). ^{13}C NMR (50 MHz, DMF-d_7): δ 47.63, 55.72, 67.02, 120.70, 126.09, 126.16, 127.15, 127.77, 128.34, 130.77, 137.25, 141.74, 144.83, 157.07, 174.19.

(S,S)-α,α'-Bis(9-fluorenylmethoxycarbonylamino)-2,3-thiophenedipropionic acid (**23a**). Dimethyl (*S,S*)-α,α'-bis(9-fluorenylmethoxycarbonylamino)-2,3-thiophenedipropionate (152 mg, 0.21 mmol) was heated in 6 M HCl (5 ml) and dioxane (5 ml) at 60 °C for 18 h. The solvents were then evaporated off and the product was purified by flash chromatography using dichloromethane–methanol–acetic acid 10:1:0.1. Residual acetic acid in the product was removed by azeotropic distillation with toluene; yield 89 mg (61%), white solid m.p. > 260 °C. ^{1}H NMR (300 MHz, DMF-d_7): δ 2.90–4.20 (m, 14 H, 4 × CH_2, 4 × CH, 2 × NH), 6.80–8.20 (m, 18 H, 16 × CH, H-4, H-5). ^{13}C NMR (75 MHz, DMF-d_7): δ 21.29, 47.83, 60.83, 61.56, 68.73, 73.73, 109.48, 120.63, 121.99, 125.98, 126.33, 127.83, 127.97, 128.25, 128.88, 129.62, 138.60, 140.62, 141.75, 144.00, 145.04. MS (electrospray): 725 (M^+ + Na), 703 (M^+ + H).

(S,S)-α,α'-Bis(9-fluorenylmethoxycarbonylamino)-5-methyl-2,3-thiophenedipropionic acid (**23b**). Dimethyl (*S,S*)-α,α'-bis(9-fluorenylmethoxycarbonylamino)-5-methyl-2,3-thiophenedipropionate (0.60 g, 0.81 mmol) was heated in 6 M HCl (12 ml) and dioxane (12 ml) at 60 °C for 18 h. The solvents were then evaporated off and the product was purified by flash chromatography using dichloromethane–methanol–acetic acid 10:1:0.1. Residual acetic acid in the product was removed by azeotropic distillation with toluene; yield 0.46 g (80%),white solid m.p. > 260 °C. MS (FAB): 717 (11, M^+ + 1), 559 (100), 291 (75).

(S,S)-α,α'-Diamino-2,5-thiophenedipropionic acid dihydrochloride (**24**). Dimethyl (*S,S*)-α,α'-diamino-2,5-thiophenedipropionate (531 mg. 1.85 mmol) was heated in 6 M HCl (30 ml) at 70 °C for 20 h, after which the solution was evaporated and the residual product dried by azeotropic distillation with benzene (2 × 30 ml). Crystalline solid; 613 mg (> 95%). ^{1}H NMR (300 MHz, D_2O): δ 3.35 (d, *J* 5.9, 4 H, 2 × CH_2), 4.16 (t, *J* 5.8 Hz, 2 H, 2 × CH), 4.65 (s, 6 H, NH_3), 6.78 (s, 2 H, H-3 and H-4). ^{13}C NMR (75 MHz, D_2O): δ 29.96 (2 × CH_2), 54.07 (2 × CH), 128.61 (C-3 and C-4), 135.89 (C-2 and C-5), 171.35 (2 × C=O).

(S,S)-α,α'-Bis(9-fluorenylmethoxycarbonylamino)-2,5-thiophenedipropionic acid (**26**). (*S,S*)-α,α'-Diamino-2,5-thiophenedipropionic acid dihydrochloride (590 mg, 1.78 mmol) was added to hexamethyldisilazane (15 ml) and trimethylsilyl chloride (1 ml). The mixture was heated under reflux overnight and the resultant solution was evaporated to dryness under reduced pressure. The residue was then dissolved in dry dichloromethane (15 ml) and the solution was cooled to 0 °C after which 9-fluorenylmethoxycarbonyl chloride (1.06 g, 4.08 mmol) in dichloromethane (5 ml) was added dropwise. The cooling bath was removed after 1 h, and the mixture stirred overnight at ambient temperature. The solvent was removed, the compound was dissolved in THF (5 ml) and 1 M HCl (5 ml) was added. The water phase was removed after 2 h and washed with chloroform (3 × 20 ml). The combined organic layers were dried ($MgSO_4$) and the solvents were evaporated off. The crude product was purified by flash chromatography using dichloromethane–methanol–acetic acid 10:1:0.1; yield 557 mg (44%), yellow crystalline material, m.p. 249 °C (decomp.). Anal. $C_{40}H_{34}N_2O_8S$: C, H. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.90–4.20 (m, 4 × CH_2, 4 × CH, 2 × NH), 6.69 (s, 2 H, H-3, H-4), 7.20–8.00 (m, 16 H, 16 × CH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 21.47, 31.50, 109.50, 120.20, 121.56, 127, 50, 129.15, 137.71, 139.83, 143.00, 172.00. MS(FAB): 703 (54, M^+ + 1), 545 (100).

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