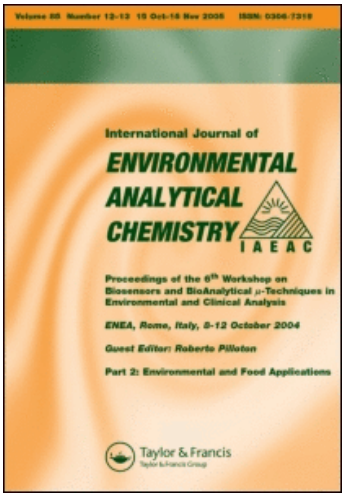

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Substituted Dibenzothiophenes I: Synthesis, Chromatography, Mass Spectrometry and Structure Elucidation by ^{1}H NMR Spectroscopy

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SUBSTITUTED DIBENZOTHIOPHENES I: SYNTHESIS, CHROMATOGRAPHY, MASS SPECTROMETRY AND STRUCTURE ELUCIDATION BY ^{1}H NMR SPECTROSCOPY

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Some polychlorinated and polymethylated dibenzothiophenes have been synthesized to serve as model compounds in environmental analysis. In order to obtain pure isomers, the synthesis mixtures have been fractionated with reversed-phase high performance liquid chromatography. In spite of a high sensitivity, mass spectrometry does not provide any reliable way to determine the precise structures of different isomers. Therefore, ^{1}H NMR spectroscopy has been utilized as an aid in their analysis. The structures for two isomeric tetramethyldibenzothiophenes could be suggested on the basis of their ^{1}H NMR spectra. Also some proposals for possible structures of two isomeric trimethyldibenzothiophenes and two fractions of chlorinated dibenzothiophenes are given. More structural isomers, however, need to be prepared for their unique structure determinations.

KEY WORDS: Methylated dibenzothiophenes, chlorinated dibenzothiophenes, reversed-phase HPLC, mass spectrometry, ^{1}H NMR spectroscopy.

INTRODUCTION

Polychlorinated dibenzothiophenes are environmentally interesting compounds due to their structural resemblance with polychlorinated dibenzodioxins and dibenzofurans[1,2]. The preparation and gas chromatographic/mass spectrometric (GC/MS) determination of some polymethylated and polychlorinated DBTs and their demethylation and dechlorination products have been previously reported[2]. Mass spectroscopy alone does not, however, provide any reliable way for an isomer-specific structure elucidation owing to the similarity of the mass spectra of the DBT isomers[2].

Reversed-phase high performance liquid chromatograph (RP-HPLC) has been commonly used to separate different isomeric mixtures and other compounds found to be difficult to separate by other analytical methods. In this work RP-HPLC was used to fractionate the synthesis mixtures found to be difficult to fractionate by other chromatographic methods.

An aim of this study is to utilize ^{1}H NMR spectroscopy in determining the structures of various DBT isomers isolated by HPLC from their synthesis mixtures[2]. Because the synthetical work had to be performed at milligram level for safety reasons and because the multistep synthesis used gave only very low yields[2], special attention

had to be paid to the selection of a proper NMR solvent and optimization of other measuring conditions.

EXPERIMENTAL

Preparation of the synthesis mixtures

The preparation of the methylated and chlorinated dibenzothiophenes has been described in the literature[2]. The compounds were prepared from chlorinated and methylated biphenyls and sulphur by Friedel-Crafts-type reactions. The yields of the required products were very low and their purification was difficult. In most cases the reaction products still were mixtures of isomers. The reaction mixtures were extracted with hexane, ethanol or toluene. Large aluminium oxide columns were used to separate the reaction products from the starting compounds and finally a carbon column with hexane/dichloromethane and toluene as eluents was used to fractionate the remaining components. However, all starting compounds could not be totally removed.

High-performance liquid chromatographic fractionation

RP-HPLC was used to fractionate the prepared polychloro- and polymethyldibenzothiophene synthesis mixtures. An HPLC system consisting of a Perkin Elmer Series 2 Liquid Chromatograph with a Shimadzu SPD GAV UV-VIS detector and a Perkin Elmer LCI-100 integrator was used. A Spherisorb S5 ODS-2 (25 cm, 0.4 mm) reversed-phase column with pre-column and acetonitrile (Rathburn, HPLC grade)-water (90:10, v/v) at 1 ml/min eluent were used for the separation. Deionized water was purified with ELGASTAT UHQ before mixing the eluent and air was removed from the eluent by ultrasonication. Detection was done by UV detection at 254 nm and all fractions were collected manually from the outlet tube from the detector. Because an analytical RP-HPLC column was used to achieve the best separation of the isomeric and other compounds each HPLC run had to be repeated 10–30 times depending on the complexity of the mixtures being fractionated. Usually the synthesis mixtures were dissolved in 200–500 μl of toluene; a loop injector of 20 μl was used.

Gas chromatographic/mass spectrometry of the fractions

Gas chromatography with MICROMAT HRGC 412 with a PID detector PI 52 02A and a SE-30 (25 m) column was done for all fractions as well as for the synthesis mixtures before fractionation with HPLC. This was done as a preliminary screening before GC/MS and ^{1}H NMR and to find the correspondence between the peaks in GC, GC/MS and HPLC. The chromatograms from a tetrachloro- and tetramethyldibenzothiophene synthesis mixture before HPLC fractionation are shown in Figs. 1 and 2. The temperature programme used was 100°C -5°C/min -200°C

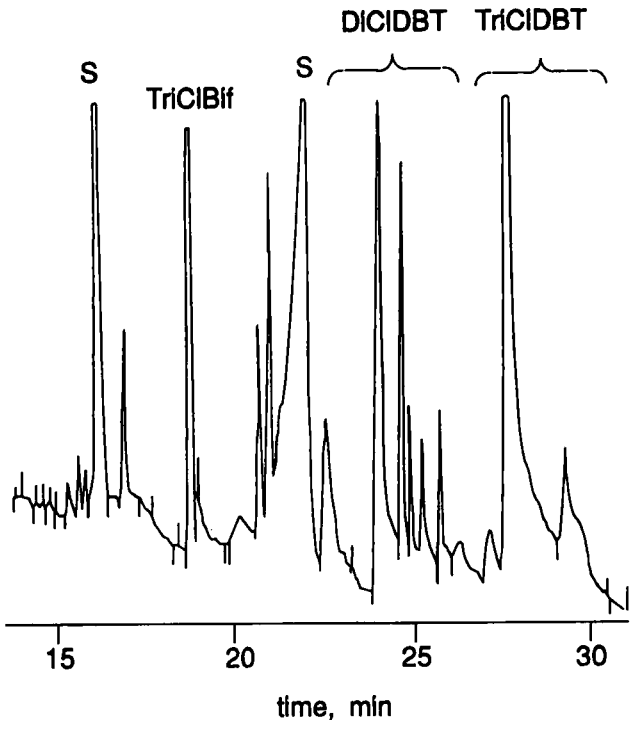


Figure 1 Gas chromatogram of the polychlorodibenzothiophene synthesis mixture before HPLC fractionation.

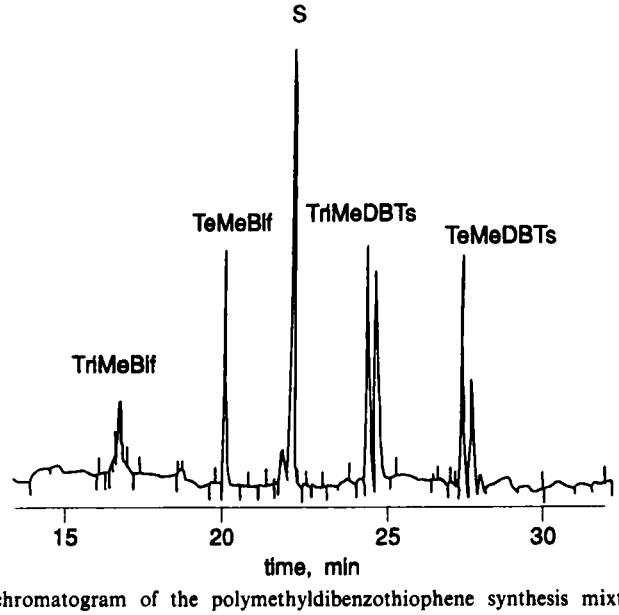


Figure 2 Gas chromatogram of the polymethyldibenzothiophene synthesis mixture before HPLC fractionation.

−10°C/min −240°C (20 min). The fractions obtained from HPLC were prepared for GC and GC/MS by evaporating most of the acetonitrile under a nitrogen flow and then extracting the chlorinated and methylated compounds with hexane. The hexane extracts were evaporated under a nitrogen flow and the compounds dissolved in 50–200 μl of toluene.

GC/MS full scan (50–500) EI mass spectra were run for all fractions and GC/MS using selected ion monitoring with molecular ions of the chlorinated dibenzothiophenes was run for some fractions supposed to contain small amounts of separate tetrachlorodibenzothiophene isomers.

A HP 5970 mass-selective detector system with a HP 5890 GC gas chromatograph and an Ultra–2 (50 m, 0.2 mm, 0.33 μm) column was used for the analysis. Helium at 21 cm/s was used as carrier gas. The temperature programme was 100°C (2 min) −20°C/min −200°C −3°C/min −300°C (20). The temperature of the injector was 250°C and that of the transfer line 300°C. The ionization potential used was 70 eV.

^1H NMR spectroscopy

Dibenzothiophene was an analytical grade product from Fluka AG, m.p. 97–99°C, and was used without further purification. CD_2Cl_2 (99.5% deuterated) was an NMR solvent from Merck (Uvasol). The substituted DBT synthesis mixtures[2,3] fractioned by RP-HPLC were dissolved in 150 μl of CD_2Cl_2 and transferred to a thick-walled (5 mm o.d./2 mm i.d.) NMR tube for ^1H NMR measurements.

All ^1H NMR spectra were measured with a Jeol GSX 270 FT NMR spectrometer working at 270.17 MHz and equipped with a standard C/H dual probe at 30°C. The spectral settings were as follows: spectral width, 2800 Hz; number of data points, 32,000 giving a digital resolution of 0.17 Hz; flip angle, 8.4 μs (90°); acquisition time, 4 s; pulse delay, 1 s; number of scans, >1000. All FIDs were exponentially windowed by a line broadening factor of digital resolution prior to Fourier transformation to improve the S/N in the frequency spectra. All chemical shifts are referenced to a solvent signal (5.3 ppm from tetramethylsilane).

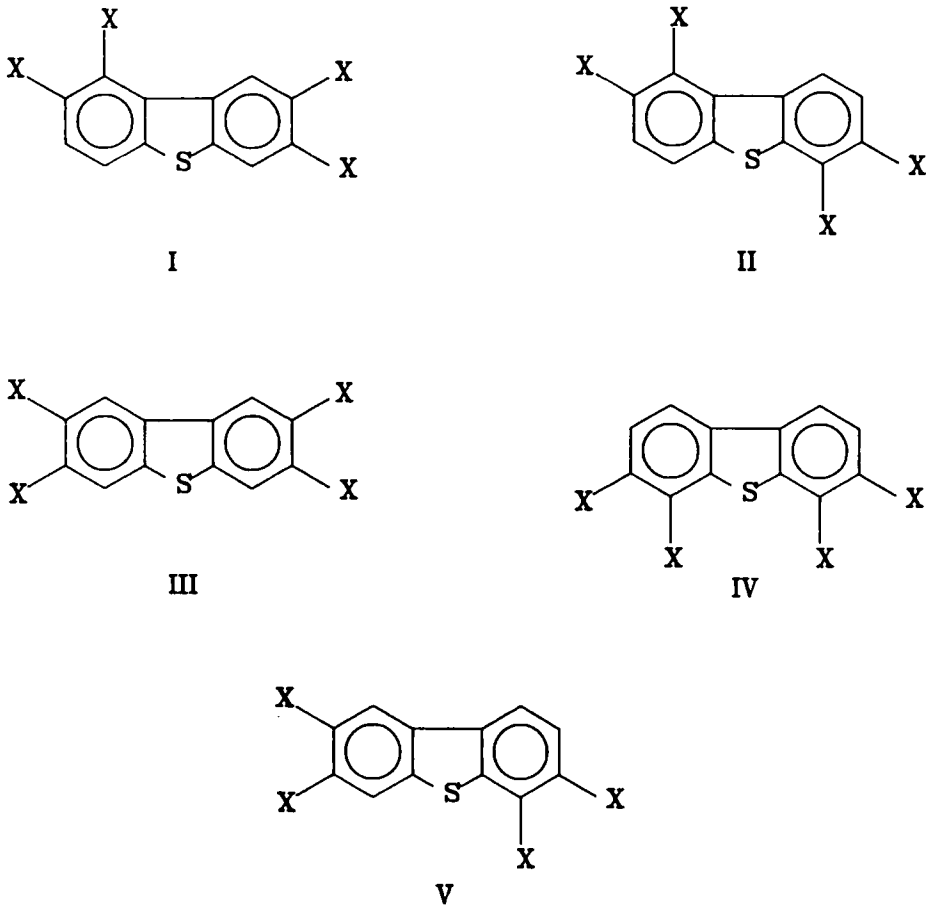
RESULTS AND DISCUSSION

Isomerism of substituted DBTs

Five possible isomers of tetrasubstituted DBTs, which can be formed in the present Friedel–Crafts-type reaction from two isomeric tetrasubstituted biphenyls[2] are shown in Scheme 1. All possible isomeric trisubstituted DBTs which can be formed via cleavage of a substituent are shown in Scheme 2.

HPLC and mass spectrometry

Figure 3 shows the HPLC chromatogram of a tetramethyldibenzothiophene synthesis mixture. Seven fractions were collected from 25 HPLC runs. The fractions 2–7 were

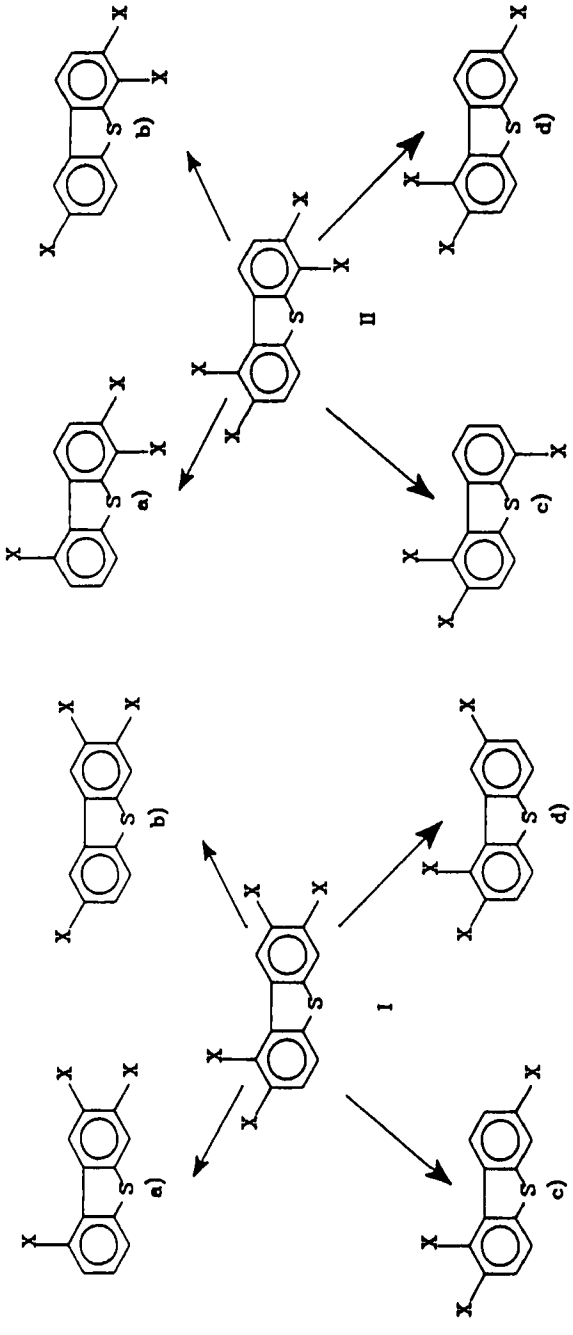


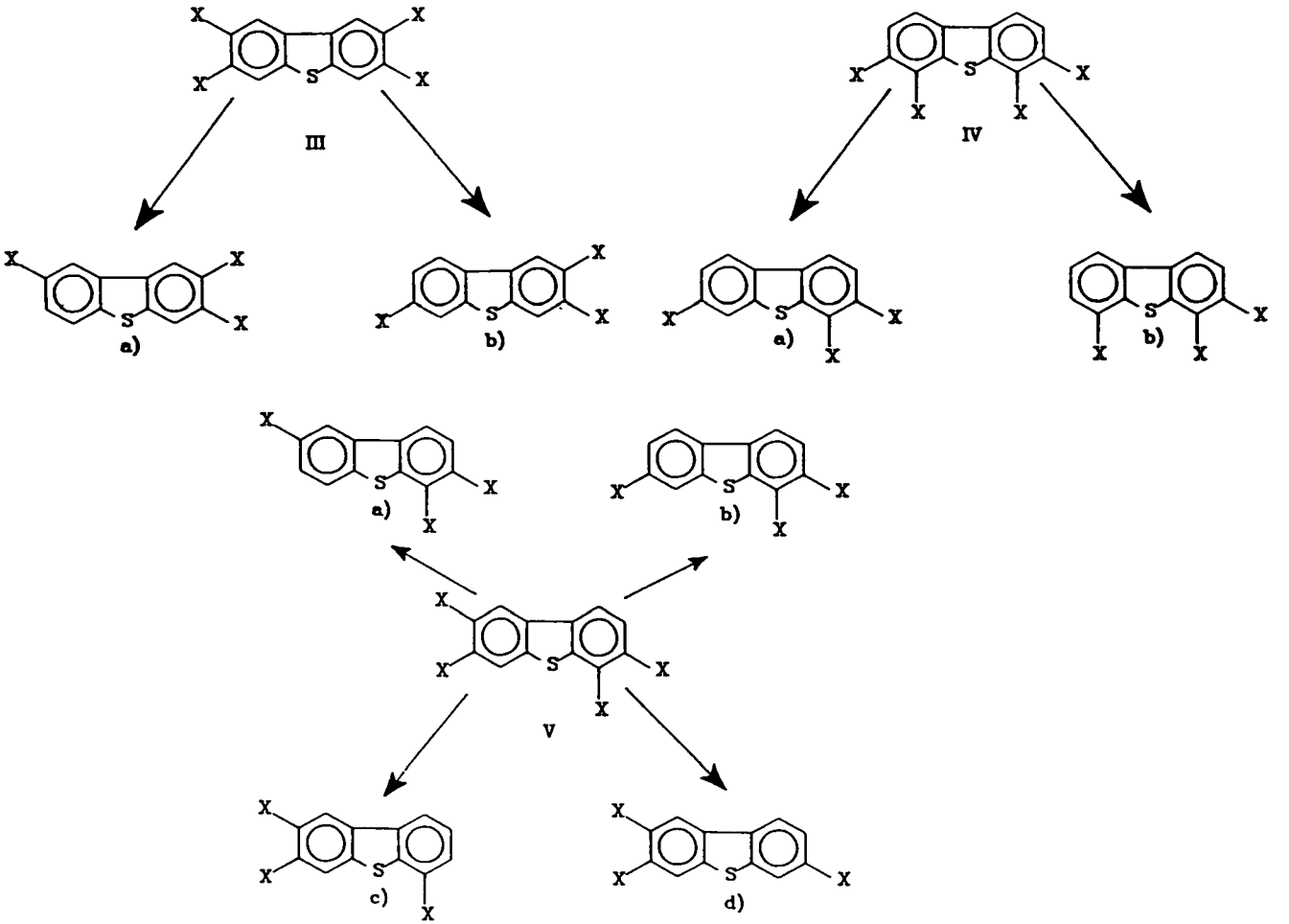
Scheme 1 The five possible isomers of tetrasubstituted DBTs, which can be formed in the reaction used to prepare the compounds.

further analysed by GC, GC/MS and ^{1}H NMR. From GC/MS it was concluded that fractions 4 and 5 each contained one trimethyldibenzothiophene isomer and fractions 6 and 7 each one tetramethyldibenzothiophene isomer. Fractions 2 and 3 each contained two trimethyldibenzothiophene isomers.

Figure 4 presents the chromatogram from a tetrachlorodibenzothiophene synthesis mixture. Ten separate fractions were collected from 10 HPLC runs. Three of the fractions collected contained at least two components (fractions 1, 3 and 5), probably different isomers of the chlorinated compounds.

According to GC/MS analysis, fraction 10 contained one trichlorodibenzothiophene and fractions 4 and 6 each one dichlorodibenzothiophene. Fraction 8 was elemental sulphur which has been used as reagent in the synthesis. The first two fractions contained trichlorobiphenyls. Fractions 3, 5, 7 and 9 could not be analysed due to very low concentrations.





Scheme 2 All possible isomeric trisubstituted DBTs, which can be formed via cleavage of a substituent.

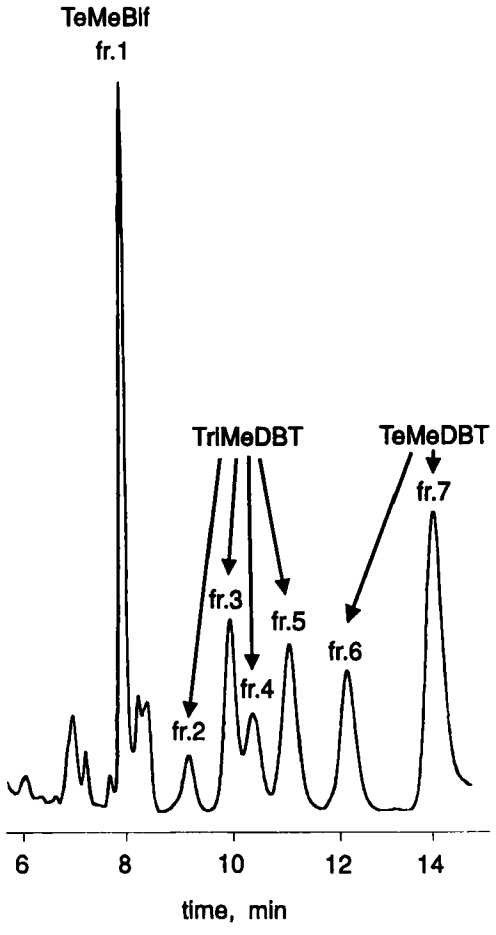


Figure 3 HPLC chromatogram of the polymethyldibenzothiophene synthesis mixture (GC chromatogram in Figure 2); eluent acetonitrile–water (90:10) at 1 ml/min; Spherisorb ODS column length, 25 cm, I.D. 0.4 mm; UV detection at 254 nm.

Some tetrachlorodibenzothiophene isomers could be separated from one synthesis mixture in low concentrations. Three late eluting HPLC fractions each contained one tetrachlorodibenzothiophene isomer.

EI mass spectra obtained from all components in the separate fractions revealed only the molecular weight of the compound from which the degree of substitution could be derived but not the exact isomeric structure.

^1H *NMR*

Figure 5 shows the ^{1}H NMR spectrum of dibenzothiophene itself. Because the inter-ring proton–proton coupling constants are vanishing, this eight-spin system is formed from two identical deceptively simple ABMX patterns. Probably for this

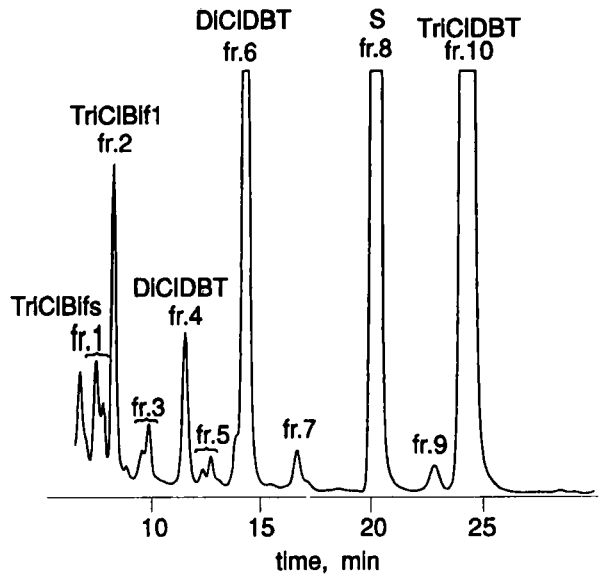


Figure 4 HPLC chromatogram of the polychlorodibenzothiophene synthesis mixture (GC chromatogram in Figure 1); eluent acetonitrile-water (90:10) at 1 ml/min; Spherisorb ODS column length, 25 cm, I.D. 0.4 mm; UV detection at 254 nm.

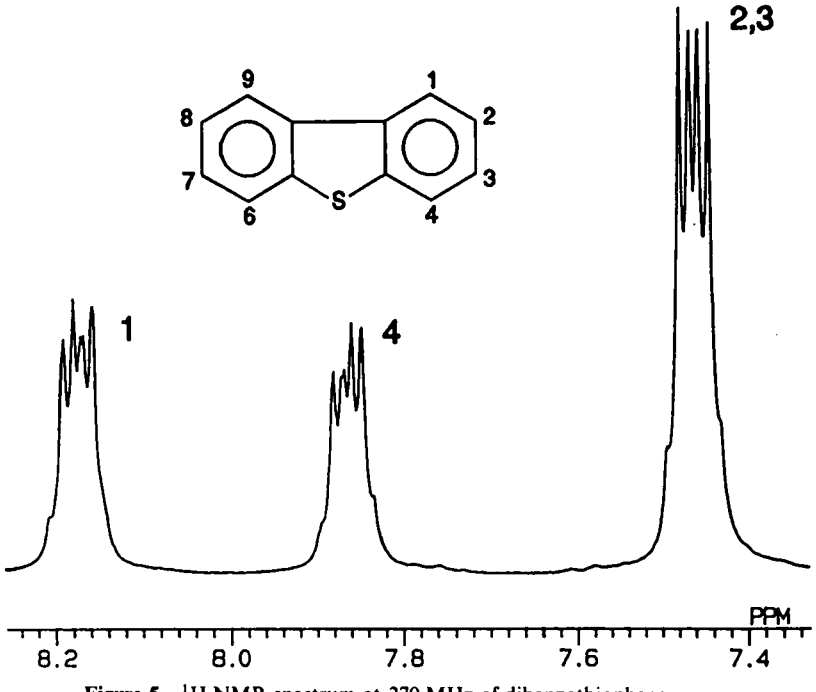


Figure 5 ^{1}H NMR spectrum at 270 MHz of dibenzothiophene.

reason, contradicting assignments of the protons 1 and 4 of DBT have been reported[4]. A computerized NMR analysis (5) of the present 270 MHz spectrum does not reveal unambiguously either the assignment of the protons 1 and 4 or if there is any difference in the chemical shifts of protons 2 and 3. Consequently some coupling constants remained inaccurate (Table 1). In substituted DBTs, this degeneracy is at least partly removed.

Interpretation of ^1H NMR spectra of MeDBTs ^1H NMR spectra of HPLC fractions 4 and 5 (trimethyl DBT) and fractions 6 and 7 (tetramethyl DBT) are shown in Figure 6. The degree of methylation of these fractions was determined on the basis of their mass spectra.

As can be observed in Figure 6, in all fractions a group of singlet resonance lines exists in the area 2.33–2.53 ppm typical for methyls bound to an aromatic ring. Additionally, all fractions give complex multiplets in the typically aromatic region of 7.2–8.0 ppm.

The NMR peaks originating from CD_2Cl_2 or its impurities at 0.8–1.6 ppm, 3.5 ppm and 5.3 ppm do not overlap with the MeDBT signals. $CDCl_3$ was also tested as an NMR solvent, but it was found to be less good than CD_2Cl_2 owing to a strong signal at ca. 7.3 ppm overlapping strongly with the aromatic signals of DBTs. Thus, CD_2Cl_2 seems to be a suitable solvent in studying methyl-substituted aromatics by ^1H NMR. A sharp solvent resonance at 5.3 ppm is used as an internal standard for chemical shifts. To diminish the amount of solvent, the NMR samples have been prepared in thick-walled NMR tubes. By this method the solvent volume can be reduced to only one fourth of that of standard 5-mm thin-walled NMR tubes.

Table 1 ^1H NMR chemical shifts (ppm) and spin-spin coupling constants (Hz) of dibenzothiophene in a 0.1 M solution in CD_2Cl_2 at 30°C

Proton	*Chemical shift (ppm)**
1	8.174
2	7.464
3	7.464
4	7.864
	Coupling constant (Hz)
J(1, 2)	7.71 ± 0.54
J(1, 3)	1.46 ± 0.54
J(1, 4)	0.62 ± 0.11
J(2, 3)	7.28 ± 0.10
J(2, 4)	0.78 ± 0.59
J(3, 4)	8.43 ± 0.59

* Accuracy of chemical shifts is better than 0.001 ppm. The accuracies of the parameters are based on the computerized analysis of the spectrum[5].

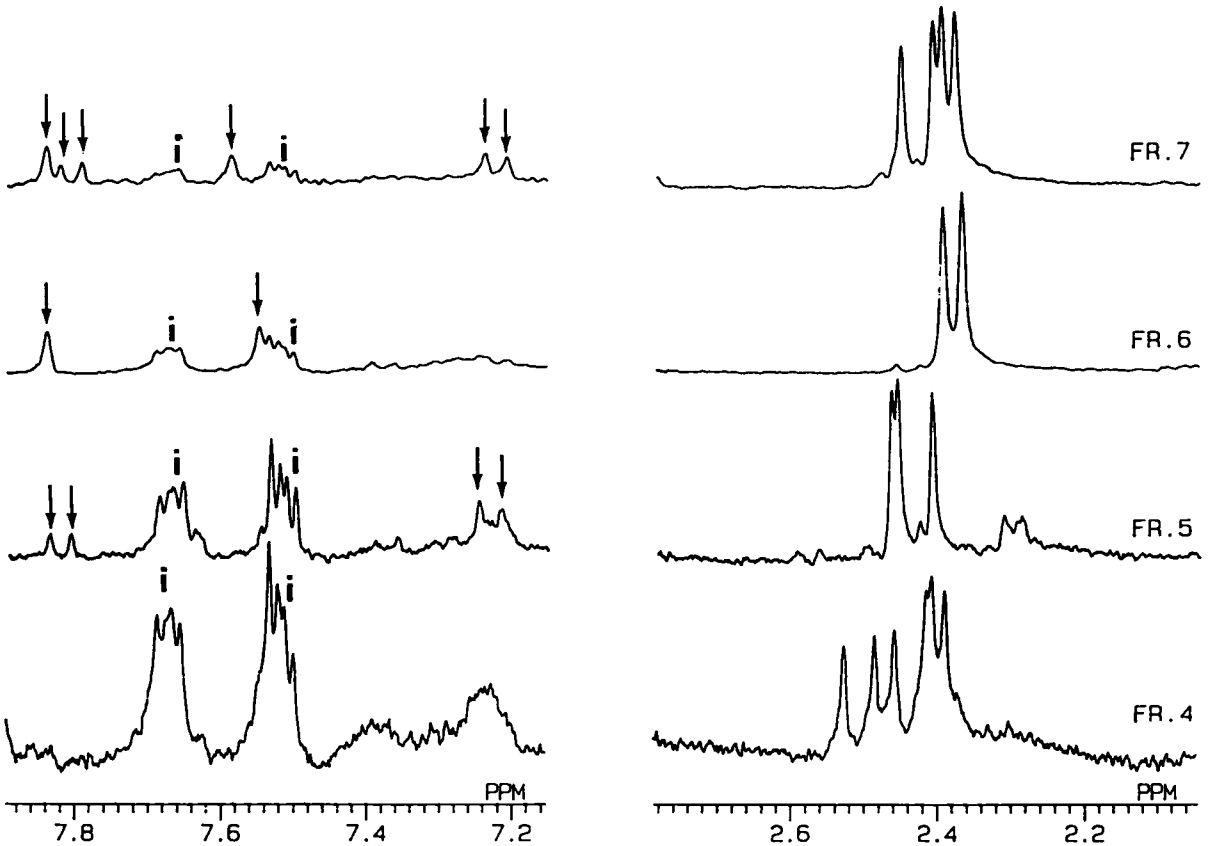


Figure 6 ^{1}H NMR spectra of HPLC fractions 4 and 5 (trimethyl-DBT) and fractions 6 and 7 (tetramethyl-DBT). Impurity signals denoted by i.

All ^{1}H NMR spectra of methylated DBTs exhibit a multiplet, in the area of 7.51–7.72 ppm, which resembles the pattern of an A_2B_2 spin system characteristic, for example, for a *para*-substituted benzene. Probably this multiplet is not caused by any DBT derivatives. It may well originate from impurities in the eluents or from the column material.

Fraction 6 (tetra-Me-DBT) shows only two signals in the methyl region, viz. at 2.38 and 2.40 ppm, which have the same intensity. This suggests that both aromatic rings should be symmetrically substituted. In the aromatic region, two strong singlet peaks occur, viz. at 7.56 and 7.85 ppm, which probably originate from the protons located at the 1,4 or *para* positions in both aromatic rings of DBT, viz. the 2,3 and 7,8 positions (isomer III). All other substituent positions described in Scheme 1 would give complex multiplets such as AB patterns in the aromatic part of the spectrum and can be excluded. Therefore, one can propose that HPLC fraction 6 is most probably 2,3,7,8-tetra-Me-DBT. Its 2,3,7,8–tetrachloro analogue will give a corresponding pattern of two singlets in the aromatic region.

Fraction 7 (tetra-Me-DBT) exhibits four signals at 2.39, 2.41, 2.42 and 2.46 ppm with the same intensity. Two of them show almost the same chemical shifts as fraction 6 (2.38 and 2.40 ppm). Therefore, one can conclude that probably one of the rings is 2,3- (or 7,8-)dimethyl substituted. This is supported by two singlets at 7.60 and 7.85 ppm in the aromatic region, very similar to what is observed for fraction 6. In addition, there exists in the aromatic region a typical AB quartet (7.22, 7.25, 7.80 and 7.83 ppm), which should originate from two adjacent (ortho) protons in the other aromatic ring. Based on the significant chemical shift difference between these protons, one can propose that one of these protons is located in the vicinity of the sulphur atom. Thus, fraction 7 is most probably 1,2,7,8-tetra-Me-DBT (isomer I).

The other HPLC fractions of tetramethylated DBTs gave ^{1}H NMR spectra with very low S/N ratios, which were not good enough to be interpreted.

Fraction 5 (tri-Me-DBT) shows three peaks: one at 2.42 ppm and two with an almost identical chemical shift of 2.47 ppm in the methyl region. Two of those are very close to two signals of fraction 7 (2.41 and 2.46 ppm). Therefore, one can propose that one of the rings is 1,2-dimethylated. Inspection of the aromatic region reveals that again an AB quartet (7.97, 7.94, 7.85 and 7.82 ppm) and a complex multiplet (7.23–7.40 ppm) show up. The AB quartet, however, differs appreciably from the AB quartet of fraction 7. Thus, based on the present spectrum the structure of this tri-Me-DBT isomer cannot be unambiguously assigned. However, the structures with two methyls in the same ring at the 2 and 3 (or 7 and 8) positions can be excluded, because two singlets due to *para* protons do not occur in the aromatic region. Thus the tetramethyl isomer III can be excluded as a parent compound for this fraction.

Fraction 4 shows six singlet resonances (2.53, 2.49, 2.46, 2.42, 2.41 and 2.39 ppm) in the methyl region. The presence of two groups of three peaks with different

intensities (5:7) reveals that the fraction is a mixture of two isomers of trimethyl DBTs. Further, neither of the two groups is similar to the methyl pattern observed in fraction 5. Therefore, both isomers of fraction 4 probably differ from that of fraction 5. The aromatic part of fraction 4 shows only poorly resolved multiplets typical for strongly second-order AB and ABC spin systems. The presence of 2,3- (or 7,8-)dimethylated rings can, however, be excluded also in this case due to lacking singlets of *para* protons.

Interpretation of ^1H NMR spectra of Cl-DBTs Is much more difficult than that of their methyl analogues, because the interpretation must be based on the aryl signals alone, for which the S/N ratio is at best 33% of that of methyl signals. In addition, only two HPLC fractions gave ^1H NMR spectra good enough to be interpreted to some extent. Based on their mass spectra, these fractions, 6 and 10, were found to be dichloro- and trichloro-DBTs, respectively.

Although ^1H NMR spectra did not reveal any unique structure for these DBT derivatives, some conclusions can be drawn. Two singlets of *para* protons are missing in both fractions, thereby excluding 2,3-dichloro substitution. In the spectrum of the tri-Cl-DBT an AB quartet with a coupling constant of 7 Hz typical for two *ortho* protons shows up. Triplet patterns typical for three adjacent aromatic protons do not appear. Consequently, the singly substituted benzene ring cannot be substituted at position 6 or 9 (10 or 4). Thus, the structure of the tri-Cl-DBT isomer could be Ic, Id, IIb (= V a), IId or IV a (= V b) (see Scheme 2).

In the di-Cl-DBT spectrum at least one triplet structure with a typically aromatic *ortho* coupling of 7 Hz exists. Thus the 2,7-, 3,7-, 2,8- and 3,8-di-Cl-DBTs can be excluded, because they cannot give the triplet pattern observed. Consequently, at least one of the benzene rings should have three adjacent aromatic patterns.

The structures of the chlorinated DBTs which have been isolated now can probably be elucidated by ^1H NMR spectroscopy, when more and more highly chlorinated isomers are available and their ^1H NMR spectra can be compared with each other.

CONCLUSIONS

Reversed-phase HPLC with an acetonitrile–water eluent was found to be successful for the fractionation of the synthesis mixtures. Several different isomers of substituted DBTs could be isolated from the mixtures by collecting fractions from repeated RP-HPLC runs.

The degree of chlorine or methyl substitution of the reaction products could be determined by GC/MS but not their precise structures. ^1H NMR spectroscopy offers an excellent method to differentiate polysubstituted DBT isomers. The greatest drawback of NMR spectroscopy, its low sensitivity in comparison with GC/MS, can be partly overcome by selecting proper solvent and measuring conditions. With the present technique the structures of DBT derivatives can be elucidated even at sub-milligram level. By using CD_2Cl_2 as a solvent it is possible to detect both methyl and aryl protons without solvent interferences.

With the tetramethylated DBTs preferred structures can be proposed for two fractions. With the trimethylated isomers the situation is more complex and only some alternative isomeric structures can be suggested for two fractions. In order to determine the structures of these isomers unambiguously more structural isomers are needed.

With chlorinated DBTs only two fractions (di- and tri-Cl-DBTs) gave ^{1}H NMR spectra good enough to be interpreted. For the trichloro fraction, five isomeric structures can be proposed. With the dichloro fraction only four isomers can be excluded and any unique structure(s) cannot be suggested. More isomers have to be made available also in this case.

Acknowledgement

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References

1. D. R. Hilker, K. M. Aldous, R. M. Smith, P. W. O'Keefe, J. F. Gierthy, J. Jurusik, S. W. Hibbins, D. Spink and R. J. Parillo, *Chemosphere*, **14**, 1275 (1985) and references cited therein.
2. S. Sinkkonen and J. Koistinen, *Chemosphere*, **21**, 1161 (1990).
3. M. Gomberg and J. C. Pernet, *J. Am. Chem. Soc.*, **28**, 1372 (1926).
4. S. Jayalakshmi, S. Perumal and D. A. Wilson, *Magn. Reson. Chem.*, **27**, 684 (1989) and references therein.
5. R. Laatikainen, *J. Magn. Reson.*, **27**, 169. (1977).