Resequencing of Comonomer Units of Well-Defined Vectra Oligomers during MALDI-TOF Mass Spectral Measurements

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ABSTRACT: To aid in the detailed characterization of the LCP polymer Vectra A950, 20 alternating dimers and 10 alternating tetrameric model oligomers were synthesized and subjected to detailed MALDI and ESI spectral characterization. The model compounds were synthesized using stepwise reactions on 4-hydroxybenzoic and 6-hydroxy-2-naphthoic acids using protection−deprotection strategies. MALDI-TOF spectra of these compounds in both the positive and negative modes were determined using dithranol, 2-(4-hydroxyphenylazo)-benzoic acid (HABA), and and its methyl ester (MHABA) as matrixes and in the presence or absence of silver trifluoroacetate. The "coolest" matrix, leading to least fragmentation, proved to be dithranol with silver trifluoroacetate, while the "hottest" was dithranol alone. On the positive side, the majority of ions detected other than the parent ions proved to be acylium ions resulting from cleavage of the unexpectedly fragile aryloxy-to-carbonyl bond. At higher applied laser power, the extrusion of oxybenzoyl groups as *p*-quinonemethide molecules from both the chain middles and ends, followed by recombination, led to extensive resequencing of the original monomer units. Similar behavior was observed in the negative ion spectra, allowance being made for the suppression of negative ions by carboxylic and phenolic functional groups. ESI spectra on the positive side, in contrast, gave much less fragmentation but more complicated spectra resulting from the occurrence of noncovalent molecular complexes. On the negative side, much fragmentation accompanied the complex spectra, reasonably resulting from the ready formation of carboxylate and phenoxide anions. In the ESI spectra, the original monomer sequences were cleanly maintained and identified. Our results demonstrate both the power and limitations of MALDI and ESI in the characterization of Vectra A950, which by implication extend to the characterization of other commercially important polymers based on 4-hydroxybenzoic acid as well.

Introduction

The characterization of highly intractable polymers such as liquid crystal polymers (LCPs) has been a challenging area of polymer research. One of these LCPs is Vectra A950, the copolymer of 4-hydroxybenzoic acid (HBA) and 6-hydroxy-2-naphthoic acid (HNA). There is no easy method to follow the progress of the polymerization or to ascertain the structures of the intermediates or side products. The reaction mixture turns heterogeneous almost immediately upon mixing due to the extreme insolubility of the growing polymer. Normal spectroscopic methods such as nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy offer very limited information about the chemical structure of the polymer.

Mass spectrometry methods, such as matrix-assisted laser desorption−ionization coupled with a time-of-flight mass analyzer (MALDI-TOF), have been used successfully for a wide variety of polymers.[1−14] MALDI-TOF analyses of soluble polymers are relatively straightforward. However, the analysis of intractable polymers such as polyamides[4,5] is more challenging, although a solventless technique[6] has proven useful for studying these polymers. The most relevant studies to Vectra include the MALDI-TOF analysis of poly-(bisphenol A) carbonate[7−9] and oligomeric poly(ethylene terephthalate).[10,11] Kricheldorf was able to obtain detection up to 55 kDa for fractionated poly(bisphenol A) carbonate.[9]

In a recent study, we successfully obtained MALDI-TOF spectra for truncated Vectra polymers and their meta analogue.[15] Pentafluorophenol and hexafluoro-2-propanol have been found to be good solvents to dissolve the polymers, and ions have been recorded up to the *m*/*z* range of 3500 Da. Some of these ions were acylium ions, indicating further truncation of the polymers presumably due to photochemical dissociation generated by the N_2 laser of the MALDI source. To further investigate and better understand this MALDI-induced fragmentation, in the present study, small model dimers and tetramers of 4-hydroxybenzoic acid (HBA) and 6-hydroxy-2-naphtholic acid (HNA) have been synthesized and investigated by MALDI-TOF mass spectrometry in both the positive and negative modes. For comparison, electrospray ionization (ESI) and tandem mass spectrometry (MS-MS) have also been used for selected compounds to investigate ion formation and fragmentation processes for these small model compounds.

In the first part of the present paper, we describe the synthesis of oligomers of HBA and HNA. This is followed by a presentation and discussion of the cations and anions detected in the MALDI-TOF spectra of these oligomers in the positive and negative modes, respectively. Three conventional matrixes were used: the "cold" matrixes 2-(4-hydroxyphenylazo)-benzoic acid (HABA) and its methyl ester (MHABA) and the "hot" matrix dithranol (2,6,7-trihydroxyanthracene, DTH).[16] Silver trifluoroacetate (CF_3COOAg) has also been used to spike the analyte with the applied matrixes. Finally, selected ESI MS and MS-MS spectra will be discussed briefly in comparison with the MALDI-TOF results.

Results and Discussion

Synthesis of Oligomers. Only a few syntheses and characterizations of well-defined LCP oligomers have been published. Volksen, Geiss, and Economy synthesized the acetyl-terminated dimer and tetramer of 4-hydroxybenzoic acid (HBA) by classical

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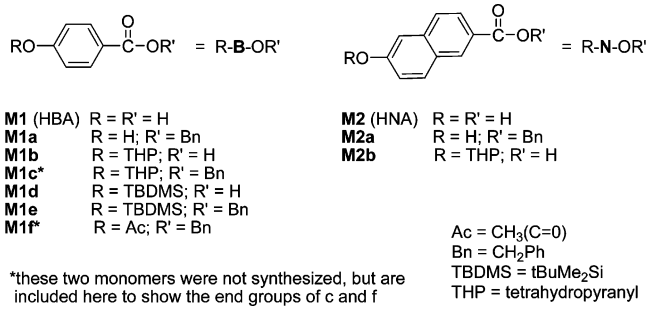
stepwise reactions.[17] Zhou, Tong, and Xie claimed the synthesis of the HBA dimer and tetramer, as well as the grafting of the latter onto styrene−maleic anhydride,[18] while Ballauf, Wu, Flory, and Barr synthesized the HBA trimer, tetramer, and pentamer.[19] In a related study, Hawker and co-workers synthesized trimers based on 4,4′-bisphenol and then functionalized them with coil segments.[20] All investigators agreed on the rapid decrease in solubility as the chain length of LCP model oligomers increased.

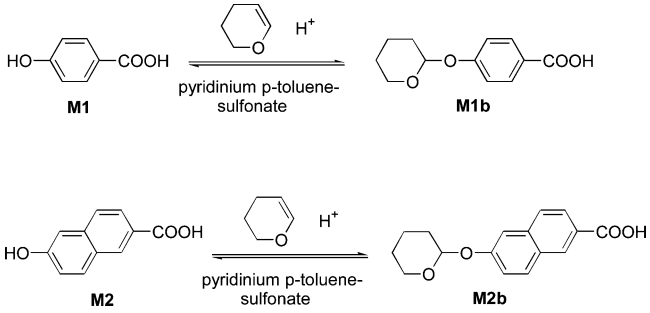
Synthesis of Monomer Intermediates. This study was limited to the synthesis of alternating oligomers of HBA and HNA. To synthesize the oligomers in a controlled fashion, protection−deprotection strategies had to be developed for the carboxylic acid and phenol functionalities of HBA and HNA.



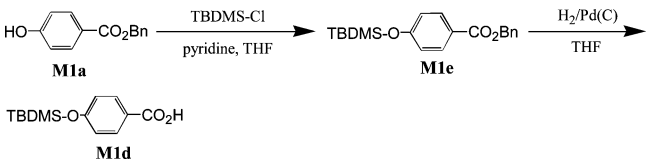
In the compound numbering scheme used in this paper: (1) **M** denotes monomer, **D** dimer, and **T** tetramer; (2) all compounds are written with the phenolic end on the left and the carboxylic end on the right; (3) the **1** series has a benzene ring on the left, while the **2** series has a naphthalene ring on the left; (4) **a**−**f** denote combinations of end groups and are used for dimers and tetramers as well as monomers.

For protection of the carboxylic acid ends, benzyl esters were chosen because deprotection can be readily accomplished by catalytic hydrogenolysis. Benzyl 4-hydroxybenzoate **M1a** is commercially available. Benzyl 6-hydroxy-2-naphthoate **M2a** was synthesized by nucleophilic displacement of potassium 6-hydroxynaphthoate on benzyl bromide in *N*,*N*-dimethylacetamide, which was much more effective than the reaction of the carboxylic acid with thionyl chloride followed by reaction with benzyl alcohol.

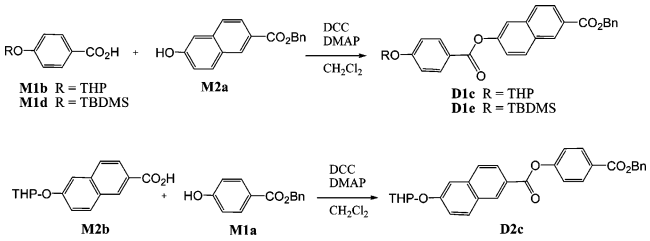
The phenolic hydroxyls were protected mostly as tetrahydropyranyl (THP) ethers. Reaction of the hydroxyacids **M1** and **M2** with 3,4-dihydro-2H-pyran in the presence of acid catalyst led to the THP-protected monomers **M1b** and **M2b** in high yield. The THP group could be removed later by refluxing with 0.25 equiv of pyridinium *p*-toluenesulfonate in THF for 20 h:



The *tert*-butyldimethylsilyl (TBDMS) group was also used to protect the phenolic hydroxyl in the benzene series. Because its direct introduction into HBA went in only about 30% yield, we prepared 4-(*tert*-butyldimethylsilyloxy)benzoic acid **M1d** by the silylation of benzyl 4-hydroxybenzoate **M1a** to its TBDMS derivative **M1e,** followed by hydrogenolysis:



Synthesis of Alternating Dimers. Couplings of the monomers to dimers with dicyclohexylcarbodiimide (DCC)/(dimethylamino)pyridine (DMAP) were found to be superior to those involving acid chlorides, although in both cases, byproducts were formed and extensive purification was needed. This route was used to prepare **D1c** from **M1b** and **M2a**, **D1e** from **M1d** and **M2a**, and **D2c** from **M2b** and **M1a**:



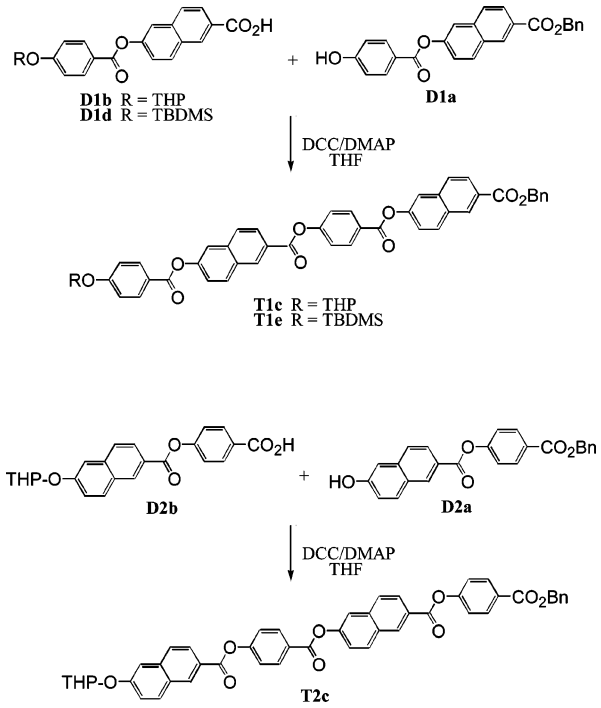
From these three dimers, eight other dimers were prepared by end group derivatization for mass spectral studies and conversion to tetramers. Hydrogenolysis using Pd/C removed the benzyl ester groups cleanly to give **D1b** from **D1c**, **D1d** from **D1e**, and **D2b** from **D2c**. The THP groups of **D1c** and **D2c** were removed with pyridinium *p*-toluenesulfonate to give phenols **D1a** and **D2a**, respectively; removal of the TBDMS group from **D1e** also gave **D1a**. Hydrogenolysis of **D1a** and **D2a** gave unprotected dimers **D1** and **D2**, respectively. Acetylation of **D2a** with acetic anhydride gave acetyl-protected dimer **D2f**.

Synthesis of Alternating Tetramers. Three tetramers were synthesized by DCC/DMAP couplings: **T1c** from acid **D1b** and phenol **D1a**, **T1e** from **D1d** and **D1a**, and **T2c** from **D2b** and **D2a**. These were the lowest-yielding steps of the whole procedure, and despite running the reactions under reflux for several days, we could only obtain about 30% yields.

For the couplings to make tetramers, only low concentrations of the reacting components could be achieved, making the reactions very slow. An attempted coupling to make an octamer was unsuccessful due to the inability to dissolve enough of the two tetramers for reaction. The tetrameric acid (**T1**) was especially insoluble. Some synthesis steps were also hampered by interchange reactions, which lowered yields and complicated reaction mixtures, necessitating difficult chromatographic separations.

From these three tetramers, seven other tetramers were prepared: Hydrogenolysis using Pd/C removed the benzyl ester groups to give **T1b** from **T1c** and **T2b** from **T2c**. The THP groups of **T1c** and **T2c** were removed with pyridinium *p*-toluenesulfonate to give phenols **T1a** and **T2a**, respectively. Hydrogenolysis of **T1a** and **T2a** gave unprotected tetramers **T1** and **T2**, respectively. Acetylation of **T1a** with acetic anhydride gave acetyl-protected tetramer **T1f**.

The syntheses of the dimers and the tetramers with alternating monomer units were successful. However, decreasing solubility of the rodlike molecules with increasing molecular length was a serious problem, in agreement with earlier reports. For example, Hawker comments on the biphenyl ester-based me-



sogenic unit, "which is known to become essentially insoluble as the size is increased to the dimer and trimer".[20]

Mass Spectral Analysis of The Oligomers. Nine dimers (**D1**; **D1a** & **D2a**; **D1b** & **D2b**; **D1c** & **D2c**; **D1e**; **D2f**) and five tetramers (**T1a**; **T1c** & **T2c**; **T1e**; **T1f**) were selected for mass spectral studies. They include four pairs of regioisomers (connected above by ampersands), which could potentially yield sequence information. 2-(4-Hydroxyphenylazo)benzoic acid (HABA), 2-(4-hydroxyphenylazo)benzoic acid methyl ester (MHABA), and dithranol (DTH) were used as MALDI matrixes in both the positive and negative ion modes. Positive ion spectra were also recorded after spiking the samples with silver trifluoroacetate CF_3COOAg. The matrixless silicon nanopartical laser desorption ionization (hereafter abbreviated as SPALDI) has also been applied for comparison. (For more details of sample preparation, see the Experimental Section.)

MALDI-TOF Mass Spectra. *Positive Ion MALDI-TOF Spectra Obtained by Spiking with (CF_3COOAg).* Cationization agents, such as alkali and Ag and Cu salts, are widely used in MALDI-TOF analysis of polymers to enhance fragmentation efficiency. For example, silver trifluoroacetate CF_3COOAg is a well-known additive compound to study the "aromatic" polystyrene. Because of the similar aromatic character of our dimers and tetramers, we carried out a set of MALDI-TOF experiments in which we spiked our analyte−matrix mixture with CF_3COOAg. As intuitively expected, the least-complicated MALDI-TOF spectra were obtained by this method. Characteristic positively charged ions obtained with the matrix dithranol are collected in Table 1, and four representative spectra are shown in Figure 1a−d. The "silverated" molecule, $[M + Ag]^+$ was detected for all of the compounds. However, some fragment ions were also detected even at low laser power (at 90% attenuation). For all the dimers, the acylium cation corresponding to the "left terminal" unit was detected at *m*/*z* 121 ($H\mathbf{B}^+$) or *m*/*z* 171 ($H\mathbf{N}^+$).[20] For the tetramers, both $H\mathbf{B}^+$ and $H\mathbf{N}^+$ ions have been observed in most cases, independently of the "left terminal" position. Other fragmentation processes include the loss of end groups, such as the loss of the THP or TBDMS groups ($[M + Ag\text{-}84]^+$ and ($[M + Ag\text{-}114]^+$, respectively) via H migration processes or the loss of the OBz group $[M\text{-}107]^+$, resulted in an acylium (not silverated) cation.

Figure 1a shows a relatively simple MALDI-TOF spectrum for the CF_3COOAg spiked **D2f** in dithranol. The $[M + Ag]^+$ ion is clearly detected (*m*/*z* 547, 549 involving the ^{107}Ag and ^{109}Ag isotopes, respectively) and only two fragment ions appear in the spectrum with significant intensity at *m*/*z* 171 ($H\mathbf{N}^+$) and 213 ($Ac\mathbf{N}^+$). Other ions are related to the dithranol matrix (denoted by M). Among those, the ion at *m*/*z* 317 is of particular interest and believed to be a reaction product with the matrix ($[Bn-DTH]^+$, see further discussion below).

The spectrum shown in Figure 1b for **D2c** indicates more efficient fragmentation even though the applied laser power was the same (90% attenuation) as that for **D2f** (Figure 1a). Although the $[M + Ag]^+$ ion is detected, fragments associated with the loss of the THP group via a H migration (*m*/*z* 505 and 507) and $H\mathbf{N}^+$ (*m*/*z* 171) are detected with significant intensity. It is interesting to note that there is a $Ag\mathbf{N}^+$ ion observed at *m*/*z* 277 and 279 although with small intensity. Other ions are related to the dithranol matrix and indicated by M.

Figure 1c for **D2b** demonstrates that, in this case, there is a competition between Ag^+ and Na^+ and K^+ to cationize the molecule: peaks at *m*/*z* 499 and 501 for $[M + Ag]^+$, *m*/*z* 415 $[M + Na]^+$, *m*/*z* 437 $[M-H + 2Na]^+$, and *m*/*z* 453 $[M-H + Na + K]^+$. Fragment ions are observed at *m*/*z* 121 ($H\mathbf{B}^+$) and 375 (the acylium $[M\text{-}OH]^+$ ion). Other ions are related to the dithranol matrix and indicated by M.

Figure 1d shows the spectrum of a tetramer (**T1e**). Despite the presence of the $[M + Ag]^+$ ion (*m*/*z* 909 and 911), several fragment ions are detected such as the silverated ion at *m*/*z* 795

Table 1. Positively Charged Ions in MALDI-TOF Spectra Obtained with the Dithranol Matrix and Spiking the Samples with CF_3COOAg

mol wt[a]		structure	MAg^+ [b]	$H\mathbf{B}^+$	$H\mathbf{N}^+$	$[MAg-X]^+$,[b,c] $[M-OY]^+$ [d]	other ions[b,e]
308	**D1**	H−**BN**−OH	415	121			
398	**D1a**	H−**BN**−OBz	505	121		−, 291	
398	**D2a**	H−**NB**−OBz	505		171		
392	**D1b**	THP−**NB**−OH	499		171	415	
392	**D2b**	THP−**BN**−OH	499	121		−, 375	
482	**D1c**	THP−**BN**−OBz	589	121		505	137
482	**D2c**	THP−**NB**−OBz	589		171	505	*277*
512	**D1e**	TBDMS−**BN**−OBz	619			−, 405	
440	**D2f**	Ac−**NB**−OBz	547		171		317
688	**T1a**	H−**BNBN**−OBz	795	121	171		
772	**T1c**	THP−**BNBN**−OBz	879	121	171	795	
772	**T2c**	THP−**NBNB**−OBz	879		171	795	
802	**T1e**	TBDMS−**BNBN**−OBz	909	121	171	795	
730	**T1f**	Ac−**BNBN**−OBz	837	121	171		137, *517*

[a] Molecular weights and all the other ion masses are in daltons. [b] For simplicity, the ions corresponding only to the ^{107}Ag isotope are indicated. [c] X denotes the THP or the TBDMS group. [d] Y = OH or OBz. [e] Ions involving Ag are in italics.

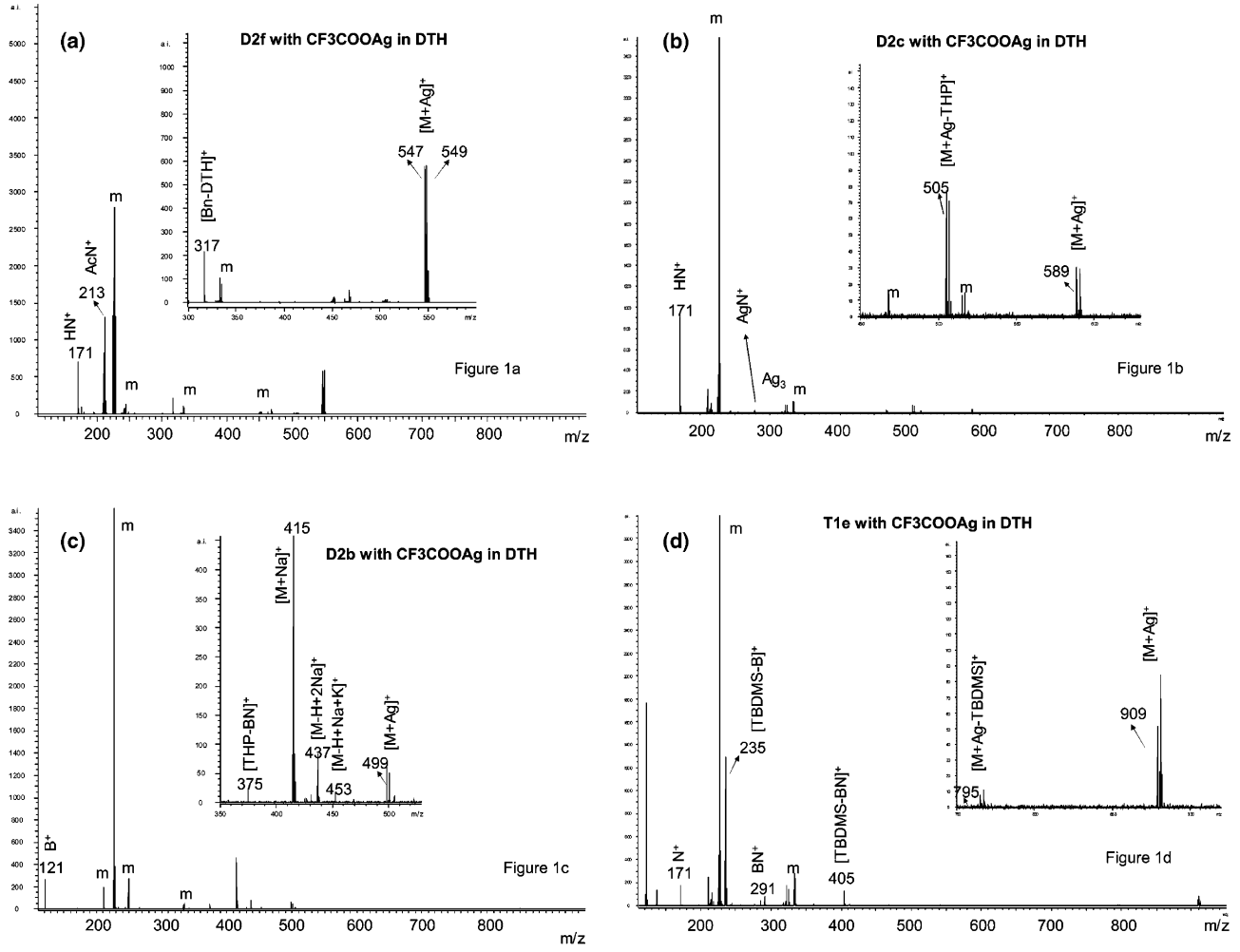


Figure 1. MALDI-TOF positive ion spectra obtained for CF_3COOAg spiked (a) **D2f**, (b) **D2c**, (c) **D2b**, and (d) **T1e**. In all cases, dithranol was used as a matrix. Ions associated with the matrix are denoted by m.

and 797 $[M + Ag-TBDMS]^+$ and other, nonsilverated ions at *m*/*z* 121 ($H\mathbf{B}^+$), 171 ($H\mathbf{N}^+$), 235 ($TBDMS-\mathbf{B}^+$), 291 ($H\mathbf{BN}^+$), and 405 ($TBDMS-\mathbf{BN}^+$). The appearance of these fragments (and also the related ones in Figure 1a−c, see also Table 1) indicates that either the molecules are just partially silverated or that even the silverated ions are not stable enough and undergo fragmentation. It is not surprising, therefore, that the MALDI-TOF spectra obtained without CF_3COOAg are much more complicated and more difficult to explain.

Positively Charged MALDI-TOF Spectra without CF_3COOAg Treatment. The *m*/*z* values of positively and negatively charged MALDI-TOF ions observed are collected in Table 2; suggested structures for the cationic and anionic peaks are shown in Tables 3 and 4, respectively. As shown in Table 2, the use of HABA resulted in sodiated ions, $[M + Na]^+$, of the dimers with essentially no fragmentation. However, the $[M + Na]^+$ ion was observed only for one tetramer (**T1e**), and there was considerable fragmentation for the rest of the tetramers, indicating that $[M + Na]^+$ ions may not be stable for Vectra-type polymers. The application of the MHABA matrix gave similar results. Dithranol (DTH, a "hotter" matrix than the other two) resulted in much more fragmentation and recombination and several cations that might originate by a reaction between the analytes and dithranol molecules.

Many of the observed cations were acyl cations, which could be formed simply by breaking one or more of the aryl ester bonds. However, other acyl cations required bond formation as well: resequencing of **B** and **N** units by recombination of the fragments in other ways can be proposed.[21] For example, the strong ion at *m*/*z* 533 from **T1c** can be related to the loss of two **B** fragments (OC_6H_4CO) from the $[M + H]^+$ species. As will be discussed below, the resulting $[THP-\mathbf{N}_2-OBn + H]^+$ cation at *m*/*z* 533 was further fragmented in ESI MS-MS spectra to give peaks at *m*/*z* 255 $[THP-\mathbf{N}]^+$ and 227 $[THP-\mathbf{N}-CO]^+$. The peak at *m*/*z* 533 in the MALDI spectra was usually accompanied by a peak at *m*/*z* 361, which is probably due to $[H-\mathbf{B}_3]^+$, showing a fate of the **B** units. Other peaks resulting from the loss of **B** fragments are at *m*/*z* 213, 341, 425, 443, 533, and 653.

Recombination of the fragments was occurring in other ways than combination of groups after loss of a **B** fragment between them. Examples are the ions at *m*/*z* 341 resulting from **D2a**, and 533 from **D1c**, respectively.

Besides the expected peaks, in the MALDI-TOF spectra of the acetyl-containing oligomers **D2f** and **T1f**, peaks at *m*/*z* 533, 653, and 773 are observed, which indicate the presence of the acetyl group (see, e.g., Figure 2a for **T1f**). Although the protonated and/or sodiated ion of **T1f** is not detected in the positive ion spectrum (Figure 2a), the $[M + Ag]^+$ ion is clearly seen in the spectrum for the CF_3COOAg spiked sample (Figure 2b). (For further evidence of the correct molecular weight, the negative $[M - H]^-$ ions for **T1f** were also detected with all the three matrixes, see Table 1.) This, together with the evidence based on NMR studies, clearly indicate that the structure of **T1f** is correct and all the positively charged ions in Figure 2a are the result of ion formation in the ionization−desorption

Table 2. Ions Observed in MALDI-TOF Spectra with HABA, MHABA, and DTH Matrixes for the Synthesized Oligomers

		HABA[b]		MHABA[b]		DTH[b]	
mol wt[a]		positive	negative	positive	negative	positive	negative
308	**D1**	H, Na	−H (w)		−H	Na, 291	−H
398	**D1a**	Na	−H	Na (w)	−H	Na (w), 317, 291	−H, 793, 623, 621, 307
398	**D2a**	Na	−H	H, Na (vw)	−H	Na, 341, 317, 291 (w)	−H, 793, 623, 621, 307
392	**D1b**	Na (w)		H (vw), Na (w)	−H	Na, 375	−H, 595, 511, 429, 307
392	**D2b**	Na (w)	−H (vw)		−H	Na (vw)	−H, 561, 307
482	**D1c**	Na		H (vw), Na (vw)		533, 375, 361, 317	621, 429, 397, 307
482	**D2c**	Na		H, Na		317	−H, 793, 623, 621, 397, 391
512	**D1e**	Na		H (vw), Na		H (w), 405, 317	−H, 421, 397
440	**D2f**	H (vw), Na (vw)		H	−H	Na, 333 (w), 317	−H, 835, 821, 793, 779, 665, 651, 623, 609, 533, 397, 349
688	**T1a**	413		653, 413	−H, 567, 397	581 (w), 317, 291	597 (vw), 567, 397
772	**T1c**	533		533, 443, 413	−H	533, 375, 361	−H, 567
772	**T2c**				−H, 397	Na, 665, 653, 425, 341, 317	−H, 793, 681, 567, 517 (w), 397
802	**T1e**	H, Na(vw)			−H (w), 567, 397	Na, 695, 405, 361, 317, 291	−H, 567, 397
730	**T1f**	773, 653, 533, 333	−H	773, 653, 533, 413	−H, 687 (vw), 567, 397	773, 653, 623, 533, 333, 213, 171, 163	−H, 677, 567, 557, 397, 387, 277, 267, 209

[a] Mol wt indicates nominal molecular weight of the compound (in dalton). [b] The following denotations are used: H = $[M + H]^+$, −H = $[M - H]^-$, Na = $[M + Na]^+$; w = weak, vw = very weak.

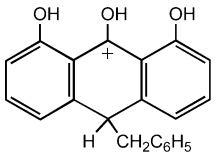
Table 3. Suggested Structures for MALDI-TOF Cations (the Sequences of B and N Groups May Be Reversed in the Structures below)[a]

mass	structure	form
163	$[Ac-\mathbf{B}]^+$	**T1f**
171	$[H-\mathbf{N}]^+$	**T1f**
213	$[Ac-\mathbf{N}]^+$	**T1f**
291	$[H-\mathbf{BN}]^+$	**D1**, **D1a**, **D2a**, **T1a**, **T1e**
317	[Bn−DTH]$^+$	all with Bn except **T1c** and **T1f**
333	$[Ac-\mathbf{BN}]^+$	**D2f**, **T1f**
341	$[H-\mathbf{N}_2]^+$	**D2a**, **T2c**
361	$[H-\mathbf{B}_3]^+$	**D1c**, **T1c**, **T1e**
375	$[THP-\mathbf{BN}]^+$	**D1b**, **D1c**, **T1c**
405	$[TBDMS-\mathbf{BN}]^+$	**D1e**, **T1e**
413	$[H-\mathbf{N}]^+$ + HABA	**T1a**, **T1c**
425	$[THP-\mathbf{N}_2]^+$	**T2c**
443	$[THP-\mathbf{N}_2-OH]H^+$	**T1c**
533	$[THP-\mathbf{N}_2-OBn]H^+$	**D1c**, **T1c**
533	$[Ac-\mathbf{B}_3-OBn]Na^+$	**T1f**
581	$[H-\mathbf{B}_2\mathbf{N}_2]^+$	**T1a**
623	$[Ac-\mathbf{B}_2\mathbf{N}_2]^+$	**T1f**
653	$[THP-\mathbf{BN}_2-OBn]H^+$	**T1a**, **T2c**
653	$[Ac-\mathbf{B}_4-OBn]Na^+$	**T1f**
665	$[THP-\mathbf{B}_2\mathbf{N}_2]^+$	**T2c**
695	$[TBDMS-\mathbf{B}_2\mathbf{N}_2]^+$	**T1e**
773	$[Ac-\mathbf{B}_5-OBn]Na^+$	**T1f**

[a] **B** denotes the benzoic acid [OC_6H_4CO], and **N** denotes the naphthoic acid [$OC_{10}H_6CO$] segments of the copolymer.

process. For another example, one can compare the MALDI-TOF spectrum of **T1e** in Figure 2c with that shown for the CF_3-COOAg spiked in Figure 1d. The absence of Ag requires the use of higher laser power, leading to more enhanced fragmentation and resequencing. For example, the ion at *m*/*z* 361 corresponding to a resequenced cyclic $[H-\mathbf{B}_3]^+$ cation is more intense in Figure 2c than in Figure 1d. For comparison and to show that those intense fragments/resequenced ions are not related to the matrix, the laser desorption ionization (LDI) spectrum of the pure matrix, MHABA, is shown in Figure 2d.

Almost all of the compounds possessing benzyl ester protecting groups gave rise to a peak at *m*/*z* 317, presumably due to a benzyldithranol cation from an analyte−matrix reaction (see Tables 1−3 and Figure 1a):



Negatively Charged MALDI-TOF and SPALDI Spectra. The 30 MALDI anions in Table 4 are suggested to be phenoxide ions (15), carboxylate ions (11), and enolate ions (4). Very weak negative ion peaks were observed in the spectra with HABA, presumably due to the presence of the acidic carboxyl group in the matrix (Table 2). The other two matrixes (MHABA and DTH), resulted in observable $[M - H]^-$ peaks for most of the oligomers. As expected, these $[M - H]^-$ peaks were much weaker for compounds that had no free hydroxyl groups. In these cases, they may be due to phenoxide ions formed after Fries rearrangement of an acyl group to an aromatic ring and loss of a proton from the resulting phenol.

The use of MHABA resulted in mostly $[M - H]^-$ ions for the dimers, but with the tetramers, other ions at *m*/*z* 397, 567, and 687 also appear in the spectra (Tables 2 and 4). These can be assigned as phenoxide ions from simple breaking of weak ester bonds. DTH resulted in many additional peaks from various reactions. The peaks at *m*/*z* 429, 533, 621, 623, 665, 793, and 835 are proposed to involve DTH matrix molecules, presumably with oxidative coupling of phenols in the case of the ion at *m*/*z* 621. Ester enolates from acetates **D2f** and **T1f** are proposed to result in the peaks at *m*/*z* 267, 387, 557, 609, 651, 665, 677, 779, 821, and 835. The series at *m*/*z* 267, 387, 557, and 677 from **T1f** is proposed to involve the potassium salts of enolized β-ketoacids and could be used to deduce the sequence of **B** and **N** units in **T1f**.

As with the cations, the preferential popping out of **B** units over **N** units was noticed. It showed in the formation of the ion at *m*/*z* 779 and in the **T2c** ion at *m*/*z* 567 (internal loss of **B**)

Table 4. Suggested Structures for MALDI-TOF Anions (the Sequences of B and N Groups May Be Reversed in the Structures below)[a]

mass	structure	from
159	Na−**B**−O$^-$	**T1f**
267	$^-$**N**−CH_2CO_2K	**T1f**
277	$^-$**N**−OBn	**T1f**
307	H−**BN**−O$^-$	all dimers except **D2c**, **D1e,** and **D2f**
349	Ac−**BN**−O$^-$	**D2f**
387	$^-$**BN**−CH_2CO_2K	**T1f**
391	THP−**BN**−O$^-$	**D1b, D2b**, **D2c**
397	$^-$**BN**−OBn	all with Bn except **T1c**
421	TBDMS−**BN**−O$^-$	**D1e**
429	THP−**B**−DTH − 2H$^+$	**D1b**, **D1c**
511	THP−$\mathbf{B_2N}$−O$^-$	**D1b**
517	$^-\mathbf{B_2N}$−OBn	**T2c**
533	H−**BN**−O$^-$ + DTH	**D2f**
557	$^-\mathbf{BN_2}$−CH_2CO_2K	**T1f**
561	THP−$\mathbf{BN_2}$−O$^-$	**D2b**
567	$^-\mathbf{BN_2}$−OBn	all 5 tetramers
595	THP−**BN**−O$^-$ + THP−**B**$^+$ − H$^+$	**D1b**
597	H−$\mathbf{B_2N_2}$−O$^-$	**T1a**
609	$^-$**N**−CH_2(CO)−**BN**−OBn	**D2f**
621	$^-$**BN**−OBn + DTH − 2H	**D1a**, **D1c**, **D2a**, **D2c**
623	$^-$**BN**−OBn + DTH	**D1a**, **D2a**, **D2c**, **D2f**
651	Ac−**N**−CH=C(O$^-$)−**BN**−OBn	**D2f**
665	CH_2=C(O$^-$)−**BN**−OBn + DTH	**D2f**
677	$^-\mathbf{B_2N_2}$−CH_2CO_2K	**T1f**
681	THP−$\mathbf{B_2N_2}$−O$^-$	**T2c**
687	$^-\mathbf{B_2N_2}$−OBn	**T1a**, **T1f**
779	$^-\mathbf{N_2}$−CH_2(CO)−**BN**−OBn	**D2f**
793	$^-\mathbf{BN_2}$−OBn + DTH	**D1a**, **D2a**, **D2c**, **D2f**, **T2c**
821	Ac−$\mathbf{N_2}$−CH=C(O$^-$)−**BN**−OBn	**D2f**
835	CH_2=C(O$^-$)−$\mathbf{BN_2}$−OBn + DTH	**D2f**

[a] **B** denotes the benzoic acid [OC_6H_4CO], and **N** denotes the naphthoic acid [$OC_{10}H_6CO$] segments of the copolymer.

being twice as big as its peak at *m*/*z* 517 (loss of **N** from the end of the chain).

To minimize complications from the matrix, silicon nanoparticle laser desorption ionization (SPALDI) experiments have also been carried out on a HNO_3 treated silica surface (see Experimental Section for further details). As intuitively expected, the positive ion mode gave less conclusive results, but we were able to obtain reasonably good spectra in the negative ion mode. This may be because it is easier to achieve either electron attachment or deprotonation for these highly aromatic and conjugated analytes. Two representative negative ion SPALDI spectra are shown in Figure 3a and b for **D1a** and **D2f**, respectively. The [M − H]$^-$ ion is detected at *m*/*z* 397 for **D1a**, together with the fragment ion at *m*/*z* 277 ([**N**−OBn]$^-$) that is related to the cleavage of the aromatic ester bond. The fragment ion at *m*/*z* 277 is observed even at the threshold laser power, although the intensity ratio of 277:397 increases with increased laser power. The ion at *m*/*z* 397 is also observed in the spectrum of **D2f**, but here, this is related to the loss of the acetyl group [M − Ac]$^-$. It is important to recognize that the anion at *m*/*z* 227 ([**B**−OBn]$^-$), originating from the cleavage of the aromatic ester bond, is more intense than the ion related to the cleavage of the acyl−ester bond cleavage (*m*/*z* 397, Figure 3b). The relative intensities of these ions are good indicators for the relative stability of the ester bonds; the para-aromatic ester bond is more fragile than that of the acetate, which is in agreement with the results discussed above for positively charged ions.

Note that the lack of the M$^-$ or [M − H]$^-$ ions is characteristic for most of the compounds investigated, and only fragments are observed in the SPALDI spectra of these dimers and tetramers. As demonstrated above, characteristic fragments are related to the cleavage of the ester bond with the charge remaining on the OBz containing moiety (see ions at *m*/*z* 277 [**N**−OBn]$^-$ for **D1a** (Figure 3a) and *m*/*z* 227 [**B**−OBn]$^-$).

Finally, we note that the ion at *m*/*z* 367 is a background ion, the origin of which is not fully explained yet.

ESI Mass Spectra. The ions generated by ESI are summarized in Table 5. Beyond the ions observed in the MALDI-TOF spectra, ions related to aggregation to noncovalent complexes are also observed. This complicates the analysis of these dimers and tetramers using the ESI ionization technique. On the other hand, ESI spectra were helpful because MS-MS could be used to identify selected ions.

Cations. Most of the oligomers gave strong [2M + Na]$^+$ peaks with isotope peaks, which in some cases showed contributions from doubly charged [4M + 2Na]$^{2+}$. In MS-MS, noncovalent clusters containing more than one M usually lost an M. Thus, in the MS-MS of the [4M + 2Na]$^{2+}$ dication of **D2f** at *m*/*z* 903, a doubly charged peak at *m*/*z* 683 was observed, indicating the loss of a neutral **D2f**. Similar loss of one M was seen from the [9M + 3Na]$^{3+}$ trimer of **D1a**, the [3M + 2Na]$^{2+}$ dications of **T1c** and **T1f**, the [3M + Na]$^+$ cation of **D1a**, and the [2M + Na]$^+$ cation of **T1f**.

Strong peaks for [M + Na + NaOH]$^+$ were observed in the mass spectra of the [2M + Na]$^+$ cations of **D1a**, **D1b**, **D2b**, **D1c**, **D2c**, and **D1e**. The largest peaks in the MS-MS spectrum of the *m*/*z* 545 cation from **D1c** were at *m*/*z* 425 from loss of a **B** unit and *m*/*z* 461 from replacement of THP by H.

In general, little fragmentation was observed in the ESI$^+$ spectra. The few fragment ions were mostly the same ones observed in MALDI-TOF spectra (Tables 1−3), *m*/*z* 653 from **T1a** and 333 and 623 from **T1f**. The [M + Na]$^+$ cation of **T1c** at *m*/*z* 795 lost the THP group to give a fragment ion at *m*/*z* 711; an ion with $^1/_7$ of the intensity of the doubly charged ion at *m*/*z* 753 showed the loss of one THP from the [2M + 2Na]$^{2+}$ dication. The [3M + H]$^+$ cation at *m*/*z* 925 from **D1** lost water to give a peak at *m*/*z* 907. Overall, the reduced fragmentation in the ESI spectra indicates that ESI is a much colder ionization technique for these oligomers than MALDI, at least with the applied laser intensities.

Anions. Negatively charged ESI ions were observed only for molecules that contained a carboxyl, phenol, or TBDMS-protected phenol. Many of the anions formed were the same ones observed under MALDI-TOF conditions (Tables 2 and 4), but additional anions came from clusters, some containing residual trifluoroacetic acid (TFA) used to clean the transfer line between samples, or its sodium salt (NaTFA). The fragment ions at *m*/*z* 277, 397, and 567 from **T1a**, the only tetramer with a free phenol end, show the possibility of sequencing the aromatic groups from this end. The corresponding TBDMS derivative **T1e** shows many of the same peaks as **T1a**; using the strongest ones would yield the correct sequence, but caution should be exercised due to resequencing, especially of the **B** units.

The MS-MS fragmentation of the *m*/*z* 391 carboxylate ion of **D2b** resulted in the following six ions in decreasing size order: *m*/*z* 307 (−THP + H), 221 (−**N**), 187 (−THP + H, −**B**), 271 (−**B**), 137 (−THP + H, −**N**), 347 (−CO_2). The MS-MS fragments of the [M−H]$^-$ phenoxide anion of **T1a** showed another possible method for sequencing of the aromatic groups in these oligomers. The peaks were at *m*/*z* 567 (100%, −**B**) and 277 (5%, −**BNB**), but no peak for loss of **BN** was observed; evidently upon helium bombardment, the phenoxide end of these oligomers, **B**, "peels off" faster than **N**.

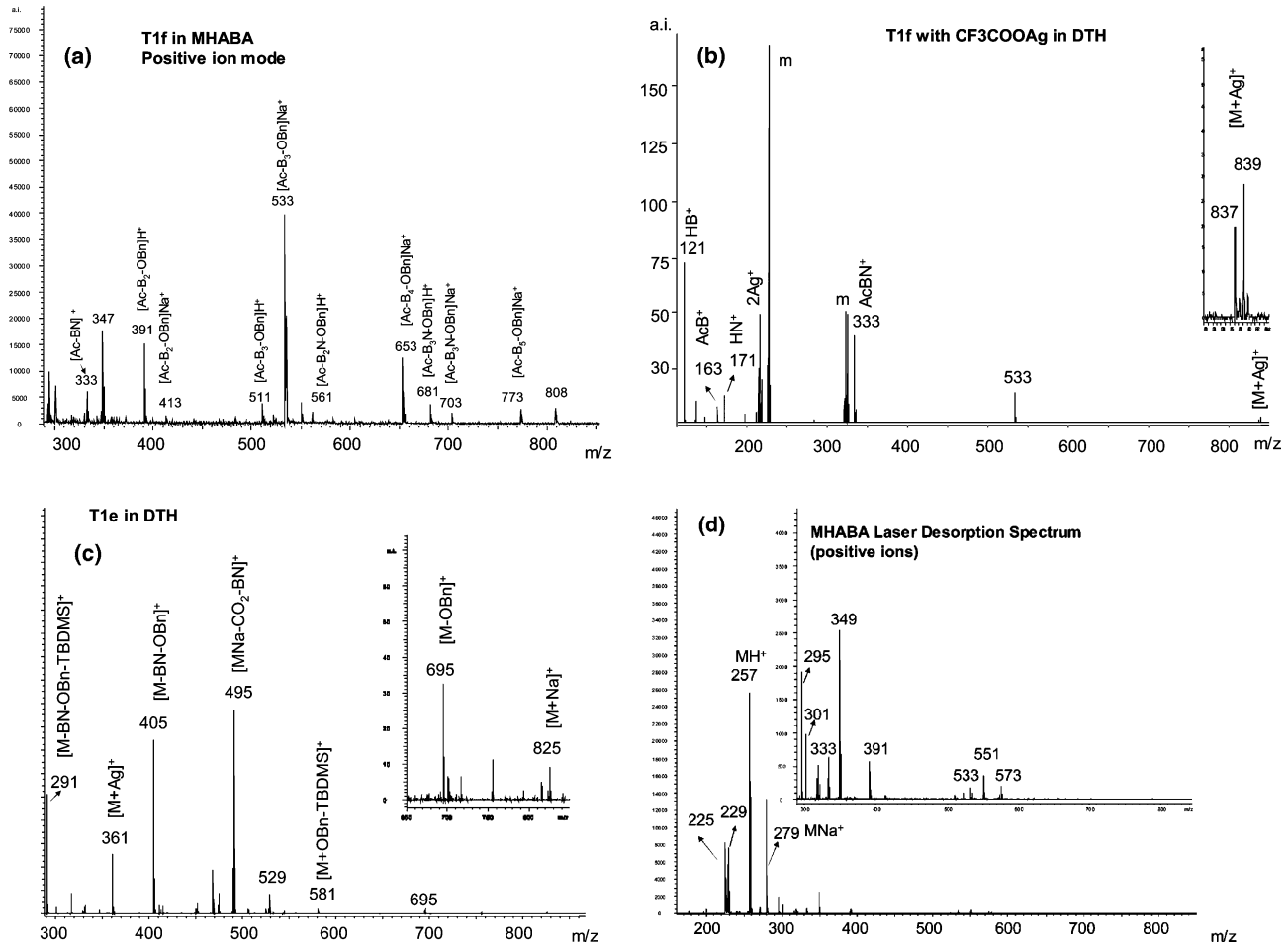


Figure 2. MALDI-TOF positive ion spectra of (a) **T1f** obtained with MHABA, (b) CF_3COOAg spiked **T1f** obtained with DTH, (c) **T1e** obtained with DTH, and (d) the laser desorption ionization (LDI) spectrum of the pure matrix, MHABA.

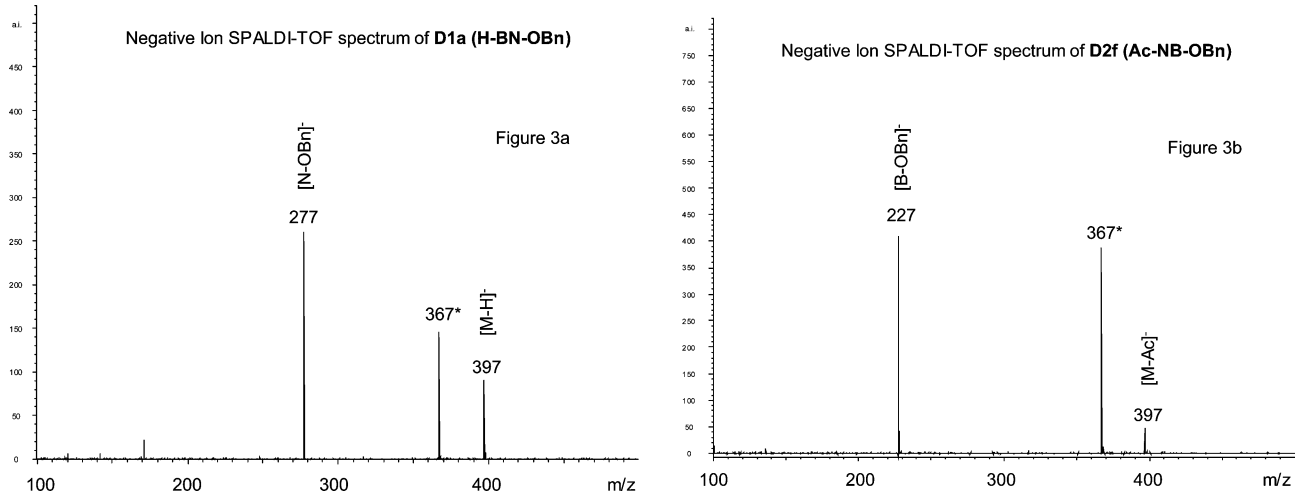


Figure 3. SELDI-TOF negative ion spectra of (a) **D1a** and (b) **D2f** (see text for details).

Conclusions

An ideal mass spectral technique for the analysis of polymers would yield a single representative peak for each molecule within the polymeric mixture. None of the methods in our study gave that kind of ideal behavior with our oligomers. The best "analytical" spectra that confirmed molecular weight information were those obtained from spiking the analytes with CF_3COOAg with the dithranol matrix. Other examples are probably the negative ion spectra, obtained with MHABA as the matrix, that show the $[M - H]^-$ anion for all the tetramers, even though there were also peaks due to other anions from fragmentations and recombinations. DTH resulted in $[M - H]^-$ ions with all but one tetramer, but much more fragmentation occurred.

Overall, the main trends in the observed fragmentation can be summarized as follows: (i) the extent of fragmentation depends on the sample preparation and the applied laser power, (ii) the internal ester bonds break more readily than the ones involving the benzyl protecting groups (see below), (iii) the resulting ions rapidly lose **B** units as neutral quinonemethide ($O{=}C_6H_4{=}C{=}O$), but not **N** units from their charged ends, (iv)

Table 5. Ions Observed in the ESI-MS Spectra with Suggested Structures

mol wt		positive	negative
308	**D1**	639 (10,[a] 2M + Na)	307 (100,[a] M − H), 615 (40, 2M − H)
398	**D1a**	461 (50, M + Na + NaOH), 819 (100, 2M + Na), 1217 (50, 3M + Na), 1615 (10, 4M + Na)	277 (25, M − H−**B**), 397 (55, M − H), 795 (15, 2M − H)
398	**D2a**	819 (100, 2M + Na), 1217 (65, 3M + Na)	397 (65, M − H), 909 (100, 2M − H + TFA[b])
392	**D1b**	455 (40, M + Na + NaOH), 807 (100, 2M + Na and 4M + 2Na), 829 (15, 2M + 2Na − H), 851 (10, 2M + 3Na − 2H), 1199 (15, 3M + Na), 1591 (3, 4M + Na)	391 (10, M − H), 527 (15, M − H + NaTFA[c])
392	**D2b**	393 (10, M + H), 455 (25, M + Na + NaOH), 807 (100, 2M + Na), 829 (25, 2M + 2Na − H), 1003 (20, 5M + 2Na), 1199 (30, 3M + Na), 1591 (10, 4M + Na)	271 (15, M − H−**B**), 391 (100, M − H), 527 (85, M − H + NaTFA), 641 (75%, M − H + NaTFA + TFA), 919 (50, 2M − H + NaTFA), 1197 (30, 3M + 2Na − 2H)
482	**D1c**	545 (80, M + Na + NaOH), 987 (100, 2M + Na), 1107 (5, 2M + Na + **B**), 1157 (1, 2M + Na + **N**), 1469 (10, 3M + Na), 1589 (1, 3M + Na + **B**), 1951 (1, 4M + Na)	no peaks
482	**D2c**	545 (75, M + Na + NaOH), 987 (100, 2M + Na), 1157 (10, 2M + Na + **N**), 1469 (25, 3M + Na), 1638 (3, 3M + Na + **N**), 1951 (2, 4M + Na)	no peaks
512	**D1e**	575 (65, M + Na + NaOH), 791 (5, 3M + 2Na), 1047 (100, 2M + Na), 1303 (5, 5M + 2Na), 1559 (20, 3M + Na)	397 (100, M−TBDMS)
440	**D2f**	463 (20, M + Na), 683 (25, 3M + 2Na), 903 (100, 2M + Na and 4M + 2Na), 1123 (10, 5M + 2Na), 1343 (30, 3M + Na and 6M + 2Na), 1563 (5, 7M + 2Na)	no peaks
688	**T1a**	1399 (2, 2M + Na)	277 (5, M −H − 2**B**−**N**), 397 (10, M − H−**B**−**N**), 567 (55, M − H−**B**), 687 (100, M − H), 801 (55, M − H + TFA), 1375 (10, 2M − H), 1489 (35, 2M − H + TFA)
772	**T1c**	533 (65, M + H − 2**B**), 795 (25, M + Na), 1181 (100, 3M + 2Na), 1567 (60, 2M + Na and 4M + 2Na), 1953 (5, 5M + 2Na)	no peaks
772	**T2c**	307 (15, H**N**$^+$ + NaTFA), 427 (65, H**BN**$^+$ + NaTFA), 653 (10, M + H−**B**)	no peaks
802	**T1e**	1627 (2, 2M + Na)	277 (30, M−TBDMS − 2**B**−**N**), 397 (35, M−TBDMS − **B**−**N**), 447 (20, M−TBDMS − 2**B**), 517 (5, M−TBDMS − **N**), 567 (100, M−TBDMS − **B**), 687 (50, M−TBDMS), 807(10, M−TBDMS + **B**), 927 (1, M−TBDMS + 2**B**)
730	**T1f**	333 (35, M − **BN** − OBn), 453 (55, M − **N** − OBn), 623 (35, M − OBn), 713 (20, M − OH), 731(40, M + H), 753 (30, M + Na), 889 (30, M + Na + NaTFA), 1118 (100, 3M + 2Na), 1178 (25, 3M + 2Na + **B**), 1483 (50, 2M + Na)	no peaks

[a] The first number in parentheses is the intensity of the peak as % of the base peak. [b] TFA = trifluoroacetic acid. [c] NaTFA = sodium trifluoroacetate.

as the neutral **B** units "pop out", cationic and anionic fragments can join, leading to the formation of **N**−**N** bonds, and finally, (v) the **B** units can protonate to give H**B**$^+$ (*m*/*z* 121), which can add further **B** units to give H**B**$_2^+$ (*m*/*z* 241) and H**B**$_3^+$ (*m*/*z* 361). The latter cation can then cyclize to the more stable isomeric carbonyl-protonated trioxacyclophane, resulting in a more intense ion at *m*/*z* 361 than those observed for the dimer and higher aggregates.

Returning to conclusion (ii), the fragility of the internal ester bonds was noticed. *p*-Hydroxybenzoic acid is incorporated in the polymer chain, resulting in *para*(−O−C_6H_4−CO−) units. Positive ion fragmentation leads to an acylium ion, which is stabilized by both the aromatic ring and the para-oxy donating substituent; negative ion fragmentation on the other hand leads to phenoxide anions (more stable than alkoxide) stabilized by the electron-withdrawing carbonyl substituent. Consistent with the present conclusions, we have previously observed an acylium ion series for truncated Vectra polymers[15] due to the fragile para-aromatic ester bond. This is in sharp contrast to the meta-isomeric analogue, also reported in our previous paper, which was much more stable.[15]

An alternative ionization method, ESI, resulted in less fragmentation than that observed in MALDI, but the ESI spectra were complicated by the occurrence of series of aggregates in almost every case. Note that ESI apparently could give better results but it cannot be considered as a primary ionization method for the MS analysis of Vectra polymers because of their very low solubility.

We believe that the MALDI results presented in this paper provide a good example of the complexity of MALDI processes occurring during the ionization−desorption previously discussed in the literature.[22] The fragility of the para-aromatic ester bond(s) in our compounds, confirmed also with the matrixless SPALDI experiments, very likely initiates reactions between analyte−analyte fragment ions and their neutral counterparts (such as O═C_6H_4═C═O), leading to "resequencing" of the copolymer units. The estimated reaction time in the plume could be up to 35−70 ns, which would be sufficiently long enough to generate complex reactions of the fragments formed in an earlier stage in the ionization process or soon after the ionization, i.e., within a time of about 2 ns.[22] The truncation and/or rearrangement of the copolymer units could certainly limit the usefulness of MALDI for the analyses of Vectra-like copolymers.

The results presented in this paper are early examples of our studies on the optimization of MALDI-TOF matrixes and sample preparation for Vectra and related polymers. More work is needed to further explore the importance of analyte−matrix ratios, the applied laser power, and the role of the cationization agent. As another avenue of investigation, noncommercial, tailor-made fluorinated matrixes are under study in our labora-

tory, the results of which will be published in a subsequent paper.

Experimental Section

Melting points were determined by DSC. NMR spectra were run at 250 MHz on a Bruker instrument, in $CDCl_3$ unless otherwise noted.

Mass Spectra and Sample Preparation. MALDI-TOF measurements were carried out on a Bruker Reflex III instrument. A conventional N_2 laser (337 nm) was operated at a pulse rate of 3 Hz with 70−90% attenuation. Reflectron-mode acquisition methods optimized for positively and negatively charged ions in the *m*/*z* 200−2500 mass range were applied. Three matrixes were used: (i) 2-(4-hydroxyphenylazo)benzoic acid (HABA), (ii) methyl 2-(4-hydroxyphenylazo)benzoate (MHABA), and (iii) dithranol (1,8,9-trihydroxyanthracene, DTH). These matrixes were dissolved in CH_2Cl_2 and/or THF to make ca. 0.1 M solutions. The oligomers were dissolved in THF at a concentration of ca. 1 mg/mL. The matrix and analyte solutions were then mixed in a 10:1 ratio, and 1 μL of this mixed solution was deposited on the MALDI plate.

ESI analysis was performed by dissolving the samples in MeOH/ACN 1:1 solvent mixture in a concentration range of about 100−200 μM and infusing these solutions with 10 μL/min rate to form the electrospray. A Thermoelectron (Finnigan) LCQ Classic instrument was used for the ESI studies. He gas was used in the MS-MS experiments with 20−30% relative collision energies.

Silicon nanoparticle laser desorption ionization (SPALDI) experiments were also carried out on the Bruker Reflex III instrument. Silicon particles (30 nm) were treated with nitric acid for oxidation, then they were derivatized with fluorinated alkyl and/or arylalkyl compounds. By applying this process, one can form a fluorinated monolayer surface that can be inert (or nonreactive) to deposit a wide variety of analytes. Our dimers and tetramers were dissolved in tetrahydrofuran and CH_2Cl_2 and were directly deposited on the treated silicon surface.

General Procedure for Ester Formation Using DCC/DMAP. The carboxylic acid (1 mmol) and phenol (1 mmol) were dissolved in DCM, and the mixture was cooled to 0 °C. DCC (1 mmol) was added, followed by DMAP (0.1 mmol), and the mixture was stirred at room temperature for 24 h, filtered, and evaporated to give the crude ester.

General Procedure for the Hydrogenolysis of Benzyl Esters. The benzyl ester was dissolved in a minimum of THF containing 10% palladium/carbon (100 mg), and the suspension was stirred under a hydrogen atmosphere until the reaction was judged complete by TLC (20−72 h). The catalyst was filtered off through a layer of Celite, and the solvent was evaporated. The residual acid was recrystallized from an appropriate solvent.

General Procedure for THP Removal. Pyridinium *p*-toluenesulfonate (0.4 equiv) was added to a THP compound dissolved in THF/ethanol (1:1) and the solution was heated at 60 °C until the reaction was judged complete by TLC (6−48 h). The solvents were removed under reduced pressure. Water (80 mL) was added to the residue, and the desired compound was extracted with DCM (3 × 100 mL). The organic phase was dried ($MgSO_4$), the solvent was removed, and the residue was purified.

*Benzyl 6-hydroxy-2-naphthoate **M2a** (85% Yield).* A mixture of 6-hydroxy-2-naphthoic acid **M2** (HNA, 18.8 g, 100 mmol), benzyl bromide (12.7 mL, 100 mmol), and potassium hydrogen carbonate (15 g, 150 mmol) in dimethylacetamide (120 mL) was stirred at room temperature overnight. The mixture was poured into water (400 mL) and extracted twice with DCM (200 mL). The combined organic layers were washed with water and brine and dried over magnesium sulfate. The solvent was evaporated, and the residue recrystallized from DCM/petroleum ether to give **M2a** as a white solid, mp 122 °C. ^{1}H NMR δ: 8.57 (1H, br s), 8.04 (1H, dd, ^3J = 8.5, ^4J = 1.5 Hz), 7.83 (1H, br d, ^3J = 8.5 Hz), 7.66 (1H, d, ^3J = 8.5 Hz), 7.36−7.52 (5H, m), 7.17 (2H, d, ^3J = 8.5 Hz), 6.15 (1H, s), 5.44 (2H, s).

*4-(Tetrahydropyran-2-yloxy)benzoic Acid **M1b** (61% Yield).* To a mixture of 4-hydroxybenzoic acid (**M1**, 27.6 g, 200 mmol), *p*-toluenesulfonic acid (1.7 g, 10 mmol) and 200 mL of diethyl ether was added to dihydropyran (27 mL) over 30 min with stirring. After stirring for 16 h, the precipitate was filtered off, washed with ether (200 mL), and then dried to yield **M1b** as a white powder, mp 116−118 °C. ^{1}H NMR δ: 8.05 (2H, d, ^3J = 9 Hz), 7.10 (2H, d, ^3J = 9 Hz), 5.53 (1H, t, ^3J = 3 Hz), 3.86 (1H, m), 3.62 (1H, m), 1.85−2.05 (3H, m), 1.58−1.77 (3H, m).

*2-(6-Tetrahydropyran-2-yloxy)naphthoic acid **M2b** (65% yield)* was prepared like **M1b** but using THF instead of diethyl ether and starting from **M2** (HNA, 18.8 g, 100 mmol), *p*-toluenesulfonic acid (0.85 g, 5 mmol), and dihydropyran (13.5 mL, 150 mmol). The reaction mixture was stirred at room temperature for 20 h and poured into water (150 mL). It was extracted with DCM (3 × 100 mL), the combined extracts were washed with water, then brine, and then dried over magnesium sulfate. The solvent was evaporated and the residue recrystallized from ether to give **M2b** as a white solid, mp 206 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.52 (1H, s), 8.03 (1H, d, ^3J = 9 Hz), 7.91 (1H, d, ^3J = 8.5 Hz), 7.84 (1H, d, ^3J = 8.5 Hz), 7.49 (1H, s), 7.30 (1H, d, ^3J = 9 Hz), 5.66 (1H, bs), 3.76 (1H, m), 3.58 (1H, m), 1.79−1.88 (3H, m), 1.55−161 (3H, m).

*4-(tert-Butyldimethylsilyloxy)benzoic Acid **M1d** (85% Yield).* A suspension of **M1e** (14 g, 41 mmol) and 10% palladium-on-carbon (50 mg) in THF (50 mL) was stirred under hydrogen for 20 h. The catalyst was filtered off, the solvent was evaporated, and the residue was recrystallized from methanol to give **M1d** as a white solid, mp 99−100 °C. ^{1}H NMR δ: 8.01 (2H, d, ^3J = 8.5 Hz), 6.88 (2H, d, ^3J = 8.5 Hz), 0.99 (9H, s), 0.24 (6H, s).

*Benzyl (4-tert-Butyldimethylsilyloxy) Benzoate **M1e** (90% Yield).* To a solution of benzyl 4-hydroxybenzoate (**M1a**, 11.41 g, 50 mmol) and imidazole (5.1 g, 75 mmol) in DCM (120 mL) cooled at 0 °C was added TBDMS−Cl (9.04 g, 60 mmol). After stirring for 20 h at room temperature, the mixture was poured into water (100 mL) and extracted with DCM (100 mL). The combined extracts were washed with water, then brine, and then dried over $MgSO_4$ and evaporated to dryness. The residue was subjected to silica gel column chromatography with methylene chloride as eluent to yield **M1e** as a colorless viscous oil. ^{1}H NMR δ: 8.00 (2H, d, ^3J = 8.5 Hz), 7.33−7.45 (5H, m), 6.87 (2H, d, ^3J = 8.5 Hz), 5.34 (2H, s), 0.99 (9H, s), 0.23 (6H, s).

*6-(4′-Hydroxybenzoyloxy)-2-naphthoic acid **D1** (62% yield)* was prepared by benzyl removal from **D1a** and recrystallized twice from ethanol as a white solid, mp 284 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.66 (1H, s), 8.21 (1H, d, ^3J = 8.5 Hz), 8.04 (2H, d, ^3J = 8.5 Hz), 8.02 (2H, bs), 7.88 (1H, s), 7.52 (1H, d, ^3J = 8.5 Hz), 6.96 (2H, d, ^3J = 8.5 Hz).

*4-(6′-Hydroxy-2′-naphthoyloxy)benzoic acid **D2** (65% yield)* was prepared by benzyl removal from **D2a** and recrystallized twice from ethanol as a white solid, mp 280 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.71 (1H, s), 8.05 (3H, d, ^3J = 8.5 Hz), 7.99 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 7.84 (1H, d, ^3J = 8.5 Hz), 7.44 (2H, d, ^3J = 8.5 Hz), 7.23 (1H, s), 7.21 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz).

*Benzyl 6-(4′-hydroxybenzoyloxy)-2-naphthoate **D1a** (75% yield)* was prepared by THP removal from **D1c** and recrystallized from ethanol as a white solid, mp 170−172 °C. This compound was also prepared in 90% yield from **D1e** by cleavage of the silyl ether: **D1e** (5.12 g, 10 mmol) was dissolved in 50 mL of THF, and a solution of tetrabutylammonium fluoride in THF (1.0 M, 10 mL) was added. The reaction was monitored by TLC, and more reagent (10 mL) was added until all of the starting material had been consumed. The mixture was acidified (1 M HCl, 50 mL) and extracted with dichloromethane (2 × 100 mL). The combined extracts were washed with water (100 mL) and brine, dried over magnesium sulfate, and evaporated. The residue was recrystallized from ethanol. ^{1}H NMR δ: 8.65 (1H, s), 8.10−8.15 (3H, m), 8.0 (1H, d, ^3J = 8.5 Hz), 7.86 (1H, d, ^3J = 8.5 Hz), 7.71 (1H, s), 7.36−7.51 (6H, m), 6.93 (2H, d, ^3J = 8.5 Hz), 5.43 (2H, s).

*Benzyl 4-(6′-hydroxy-2′-naphthoyloxy) benzoate **D2a** (73% yield)* was prepared by THP removal from **D2b**, chromatographed on silica gel (DCM/EtOAc: 9:1), and recrystallized from ethanol as a white

solid, mp 162 °C. ^{1}H NMR δ: 8.70 (1H, s), 8.18 (2H, d, ^3J = 8.5 Hz), 8.12 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 7.89 (1H, d, ^3J = 8.5 Hz), 7.74 (1H, d, ^3J = 8.5 Hz), 7.33−7.48 (7H, m), 7.16−7.21 (2H, m), 5.39 (2H, s).

6-(4′-Tetrahydropyran-2-yloxybenzoyloxy)-2-naphthoic acid ***D1b*** *(82% yield)* was prepared by benzyl removal from **D1c** (reaction time 72 h) and recrystallized from methanol as a white solid, mp 270 °C (dec). ^{1}H NMR δ: 8.73 (1H, s), 8.20 (2H, d, ^3J = 8.5 Hz), 8.15 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 8.04 (1H, d, ^3J = 8.5 Hz), 7.90 (1H, d, ^3J = 8.5 Hz), 7.76 (1H, bs), 7.45 (1H, dd, ^3J = 8.5 Hz and ^4J = 2 Hz), 7.17 (2H, d, ^3J = 8.5 Hz), 5.58 (1H, bs), 3.89 (1H, m), 3.66 (1H, m), 1.92−2.04 (3H, m), 1.62−1.71 (3H, m).

4-(6′-Tetrahydropyran-2-yloxy-2′-naphthoyloxy)benzoic acid ***D2b*** *(90% yield)* was prepared by benzyl removal from **D2c** and recrystallized from CH_2Cl_2/methanol as a white solid, mp 265 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.77 (1H, s), 8.16 (1H, d, ^3J = 8.5 Hz), 8.05 (1H, d, ^3J = 8.5 Hz), 7.95 (1H, d, ^3J = 8.5 Hz), 7.55 (1H, s), 7.44 (2H, d, ^3J = 8.5 Hz), 7.36 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 5.70 (1H, bs), 3.77 (1H, m), 3.61 (1H, m), 1.80−1.90 (3H, m), 1.55−1.63 (3H, m).

Benzyl 6-(4′-tetrahydropyran-2-yloxybenzoyloxy)-2-naphthoate ***D1c*** *(67% yield)* was prepared from **M1b** with **M2a**, purified by column chromatography on silica gel (DCM), and recrystallized from ethanol as a white solid, mp 116−118 °C. ^{1}H NMR δ: 8.66 (1H, br s), 8.19 (2H, d, ^3J = 8.5 Hz), 8.12 (1H, d, ^3J = 8.5 Hz), 8.0 (1H, d, ^3J = 8.5 Hz), 7.86 (1H, d, ^3J = 8.5 Hz), 7.72 (1H, s), 7.37−7.49 (6H, m), 5.64 (1H, bs), 5.39 (2H, s), 3.94 (1H, m), 3.69 (1H, m), 1.98−2.1 (3H, m), 1.65−1.75 (3H, m).

Benzyl 4-(6′-tetrahydropyran-2-yloxy-2′-naphthoyloxy)benzoate ***D2c*** *(63% yield)* was prepared from **M2b** and **M1a**, purified by silica gel chromatography (DCM/EtOAc, 95:5), and recrystallized from EtOH as a white solid, mp 118 °C. ^{1}H NMR δ: 8.70 (1H, br s), 8.19 (2H, d, ^3J = 8.5 Hz), 8.14 (1H, dd, ^3J = 8.5 Hz, ^4J = 2 Hz), 7.91 (1H, d, ^3J = 8.5 Hz), 7.83 (1H, d, ^3J = 8.5 Hz), 7.72 (1H, s), 7.31−7.48 (9H, m), 7.16 (2H, d, ^3J = 8.5 Hz), 5.57 (1H, bs), 5.44 (2H, s), 3.84−3.92 (1H, m), 3.62−3.68 (1H, m), 1.92−2.02 (3H, m), 1.61−1.70 (3H, m).

6-(4′-tert-Butyldimethylsilyloxybenzoyloxy)-2-naphthoic acid ***D1d*** *(85% yield)* was prepared by benzyl removal from **D1e** and recrystallized from DCM as a white solid, mp 180 °C (dec). ^{1}H NMR δ: 8.71 (1H, s), 8.10−8.19 (3H, m), 8.04 (1H, d, ^3J = 8.5 Hz), 7.86 (1H, d, ^3J = 8.5 Hz), 7.77 (1H, s), 7.45 (1H, d, ^3J = 8.5 Hz), 7.13 (2H, d, ^3J = 8.5 Hz), 1.01 (9H, s), 0.27 (6H, s).

Benzyl 6-(4′-tert-butyldimethylsilyloxybenzoyloxy)-2-naphthoate ***D1e*** *(55% yield)* was prepared from **M1d** and **M2a**, purified by silica gel chromatography (DCM/petroleum ether, 1:1), and recrystallized from petroleum ether as white crystals, mp 82 °C. ^{1}H NMR δ: 8.67 (1H, s), 8.15 (2H, d, ^3J = 9 Hz), 8.12 (1H, dd, ^3J = 9 Hz and ^4J = 1.5 Hz), 8.01 (1H, d, ^3J = 9 Hz), 7.86 (1H, d, ^3J = 9 Hz), 7.72 (1H, s), 7.34−7.52 (6H, m), 6.96 (2H, d, ^3J = 9 Hz), 5.44 (2H, s), 1.01 (9H, s), 0.27 (6H, s).

Benzyl 4-(6′-Acetoxy-2′-naphthoyloxy)benzoate ***D2f*** (93% *Yield)*. A mixture of **D2a** (200 mg, 2.2 mmol) and pyridine (2 mL) in acetic anhydride (10 mL, excess) was refluxed overnight and poured into cold water (50 mL) with stirring. The mixture was stirred until hydrolysis of the excess Ac_2O was complete (ca. 30 min) and extracted with $CHCl_3$ (4 × 40 mL). The combined organic extracts were washed with water (50 mL) and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from EtOH to give **D2f** as a white solid. ^{1}H NMR δ: 8.78 (1H, s), 8.21 (3H, m), 8.02 (1H, d, ^3J = 9 Hz), 7.91 (1H, d, ^3J = 9 Hz), 7.66 (1H, d, ^4J = 2 Hz), 7.35−7.48 (8H, m), 5.39 (2H, s), 2.39 (3H, s).

[6″-(4‴-Hydroxybenzoyloxy)-4′-(2″-naphthoyloxy)-6-benzoyloxy]-2-naphthoic acid ***T1*** *(56% yield)* was prepared by benzyl removal from **T1a** and recrystallized twice from a mixture of THF/ethanol as an off-white solid, mp 283−285 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.94 (1H, s), 8.73 (1H, s), 8.26−8.30 (4H, m), 8.14 (2H, d, ^3J = 8.5 Hz), 7.96−8.05 (6H, m), 7.33−7.41 (4H, m), 6.97 (2H, d, ^3J = 8.5 Hz).

[4″-(6‴-Hydroxy-2‴-naphthoyloxy)-6′-benzoyloxy-4-(2′-naphthoyloxy)]benzoic acid ***T2*** *(37% yield)* was prepared by benzyl removal from **T2a** and recrystallized twice from ethanol as a yellowish solid, mp 260 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.96 (1H, s), 8.76 (1H, s), 7.86−8.31 (11H, m), 7.48−7.60 (5H, m), 7.22 (2H, bs).

Benzyl [6″-(4‴-hydroxybenzoyloxy)-4′-(2″-naphthoyloxy)-6-benzoyloxy]-2-naphthoate ***T1a*** *(65% yield)* was prepared by THP removal from **T1c** and recrystallized twice from ethanol as a white solid, mp 223 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.94 (1H, s), 8.73 (1H, s), 8.26−8.30 (4H, m), 8.14 (2H, d, ^3J = 8.5 Hz), 7.96−8.05 (6H, m), 7.36−7.63 (9H, m), 6.96 (2H, d, ^3J = 8.5 Hz), 5.42 (2H, s).

Benzyl [4″-(6‴-hydroxy-2‴-naphthoyloxy)-6′-benzoyloxy-4-(2′-naphthoyloxy)]benzoate ***T2a*** *(71% yield)* was prepared by THP removal from **T2c** and recrystallized from ethanol as a white solid, mp 230 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.94 (1H, s), 8.73 (1H, s), 8.40 (1H, d, ^3J = 8.5 Hz), 8.13−8.24 (5H, m), 7.94−8.01 (2H, m), 7.82−7.89 (2H, m), 7.39−7.52 (13H, m), 5.39 (2H, s).

[6″-(4‴-Tetrahydropyran-2-yloxybenzoyloxy)-4′-(2″-naphthoyloxy)-6-benzoyloxy]-2-naphthoic acid ***T1b*** *(31% yield)* was prepared by benzyl removal from **T1c** and recrystallized twice from a mixture of THF/ethanol as an off-white solid, mp 273−275 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.83 (1H, s), 8.67 (1H,s), 8.35 (2H, d, ^3J = 8.5 Hz), 8.21−8.25 (3H, m), 8.16(1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 8.01−8.12 (2H, m), 7.94 (1H, d, ^3J = 8.5 Hz), 7.88 (1H, d, ^3J = 8.5 Hz), 7.77 (2H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 7.37−7.46 (4H, m), 7.18 (2H, d, ^3J = 8.5 Hz), 5.58 (1H, bs), 3.90−3.98 (1H, m), 3.66−3.72 (1H, m), 1.94−2.06 (3H, m), 1.63−1.73 (3H, m).

[4″-(6‴-Tetrahydropyran-2-yloxy-2‴-naphthoyloxy)-6′-benzoyloxy-4-(2′-naphthoyloxy)]benzoic acid ***T2b*** *(53% yield)* was prepared by benzyl removal from **T2c** and recrystallized twice from ethanol as a white solid, mp 245 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 8.95 (1H, s), 8.74 (1H, s), 7.85−8.29 (12H, m), 7.48−7.60 (4H, m), 7.22 (2H, d, ^3J = 8.5 Hz), 5.69 (1H, bs), 5.43 (2H, s), 3.90−3.99 (1H, m), 3.67−3.74 (1H, m), 1.95−2.08 (3H, m), 1.64−1.76 (3H, m).

Benzyl [6″-(4‴-tetrahydropyran-2-yloxybenzoyloxy)-4′-(2″-naphthoyloxy)-6-benzoyloxy]-2-naphthoate ***T1c*** *(32% yield)* was prepared from **D1b** and **D1a** and recrystallized twice from ethanol as a white solid, mp 228−232 °C. ^{1}H NMR δ: 8.83 (1H, s), 8.68 (1H,s), 8.36 (2H, d, ^3J = 8.5 Hz), 8.23 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 8.21 (1H, d, ^3J = 8.5 Hz), 8.11 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 8.07 (1H, d, ^3J = 8.5 Hz), 8.03 (2H, d, ^3J = 8.5 Hz), 7.95 (1H, d, ^3J = 8.5 Hz), 7.88 (1H, d, ^3J = 8.5 Hz), 7.79 (2H, d, ^4J = 1.5 Hz), 7.77 (2H, d, ^4J = 1.5 Hz), 7.34−7.53 (9H, m), 7.18 (2H, d, ^3J = 8.5 Hz), 5.60 (1H, bs), 5.45 (2H, s), 3.92 (1H, m), 3.69 (1H, m), 1.94−2.06 (3H, m), 1.63−1.73 (3H, m).

Benzyl [4″-(6‴-tetrahydropyran-2-yloxy-2‴-naphthoyloxy)-6′-benzoyloxy-4-(2′-naphthoyloxy)]benzoate ***T2c*** *(33% yield)* was prepared from **D2b** and **D2a** and recrystallized twice from ethanol, mp 244−246 °C. ^{1}H NMR δ: 8.82 (1H, s), 8.76 (1H, s), 8.36 (1H, d, ^3J = 8.5 Hz), 8.07−8.21 (5H, m), 7.91−7.97 (2H, m), 7.80−7.86 (2H, m), 7.36−7.49 (13H, m), 5.65 (1H, bs), 5.39 (2H, s), 3.90−3.98 (1H, m), 3.66−3.72 (1H, m), 1.94−2.06 (3H, m), 1.63−1.73 (3H, m).

Benzyl [6″-(4‴-tert-butyldimethylsilyloxybenzoyloxy)-4′-(2″-naphthoyloxy)-6-benzoyloxy]-2-naphthoate ***T1e*** *(31% yield)* was prepared from **D1d** and **D1a** and recrystallized twice from ethanol as a white solid, mp 240 °C (dec). ^{1}H NMR δ: 8.84 (1H, s), 8.68 (1H, s), 8.36 (2H, d, ^3J = 8.5 Hz), 8.23 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 8.17 (2H, d, ^3J = 8.5 Hz), 8.14 (1H, dd, ^3J = 8.5 Hz and ^4J = 1.5 Hz), 8.36 (2H, d, ^3J = 8.5 Hz), 8.03 (2H, d, ^3J = 8.5 Hz), 7.96 (1H, d, ^3J = 8.5 Hz), 7.88 (1H, d, ^3J = 8.5 Hz), 7.79 (1H, d, ^4J = 1.5 Hz), 7.77 (1H, d, ^4J = 1.5 Hz), 7.34−7.52 (9H, m), 6.97 (2H, d, ^3J = 8.5 Hz), 5.44 (2H, s), 1.02 (9H, s), 0.28 (6H, s).

Benzyl [6″-(4‴-Acetoxybenzoyloxy)-4′-(2″-naphthoyloxy)-6-benzoyloxy]-2-naphthoate ***T1f*** *(92% Yield).* A mixture of **T1a** (200 mg, 0.29 mmol) and pyridine (2 mL) in acetic anhydride (10 mL,

excess) was refluxed overnight and poured into cold water (50 mL) with stirring. The mixture was stirred until hydrolysis of the excess Ac_2O was complete (ca. 30 min) and extracted with $CHCl_3$ (4 × 40 mL). The combined organic extracts were washed with water (50 mL) and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from EtOH to give compound **T1f** as a white solid, mp 219 °C. ^{1}H NMR (DMSO-d_6) δ: 8.76 (1H, s), 8.59 (1H, s), 8.15−8.30 (4H, m), 7.79−8.07 (6H, m), 7.70 (2H, d, ^3J = 8 Hz), 7.35−7.41 (9H, m), 7.21 (2H, d, ^3J = 8 Hz), 5.36 (2H, s), 2.28 (3H, s).

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