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CIRCULAR DICHROISM SPECTRA OF BICHROMOPHORICALLY DERIVATIZED METHYL-D-GALACTOPYRANOSIDES, CALCULABLE BY PAIRWISE ADDITIVITY, PROVIDE A BASIS FOR NOVEL MICRO-ANALYSIS OF OLIGOSACCHARIDES

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ABSTRACT

The spectroscopic basis for a novel alternative to methylation microanalysis for linkage determination is presented. The complex c.d. spectra of "bichromophoric" D-galactopyranoside derivatives, *i.e.*, containing two types of exciton-coupling chromophore, namely, *p*-bromobenzoate (λ_{max} 245 nm) and *p*-methoxycinnamate (λ_{max} 311 nm), are highly characteristic at nanomolar levels, indicative of the sugar, the substitution pattern, and the D or L configuration. That these spectra are due to a recently demonstrated pairwise additivity is confirmed. Work directed towards an oligosaccharide derivatization-sequence, resulting in the easily identifiable tetrachromophoric monosaccharide residues, is described. Such an analysis can simultaneously accomplish identification of sugar components, linkage pattern, and determination of absolute configuration at the nanomolar level.

INTRODUCTION

Methylation analysis has for many years been the standard method for determination of glycosidic linkage of oligosaccharides. Whereas the derivatization sequence to provide partially methylated alditol acetates for g.l.c.–m.s. generally requires a minimum of 25 nmol of material, a capillary g.l.c.–c.i.m.s. study has been carried out at the subnanomolar level[1]. At this level, however, assignments were based only upon retention times and molecular weight, and therefore[1] "should preferably be used in conjunction with other analytical techniques which corroborate the results". Certain ambiguities in linkage position (*i.e.*, both 4-linked aldohexopyranose and 5-linked aldohexofuranose residues yield 4,5-di-*O*-acetylhexitols) can be resolved *via* the alternative reductive-cleavage method[2–4]. All such analyses suffer the disadvantage the relying upon comparisons of retention time for a large number of authentic samples which must be prepared synthetically[3–5]. These analyses result in destruction of the sample, another significant dis-

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advantage considering the need for complementary analyses for sugar identification and for determination of the D or L configuration of the monosaccharide residues.

An alternative technique for linkage analysis based upon circular dichroism (c.d.) spectroscopy of monosaccharide fragments derivatized with exciton-coupling chromophores would not suffer from the shortcomings of conventional methylation. We have recently found[6] that the complex c.d. spectra of "bichromophoric" D-glucopyranoside derivatives, *i.e.*, those containing two different *types* of chromophore, namely, *p*-bromobenzoate and *p*-methoxycinnamate, result from the summation of all degenerate and nondegenerate *pairwise* interactions between the chromophores. Pairwise additivity is confirmed here for the entire analogous series of methyl α-galactopyranoside derivatives. More importantly, the complex, and yet easily interpretable, c.d. curves of these bichromophoric derivatives are shown to be highly characteristic of both the substitution pattern and the sugar, as well as reflecting the D or L configuration, and thus, they form the basis for a highly sensitive microanalysis for simultaneous determination of glycosidic linkage and sugar component.

RESULTS

All permutations of tetrachromophoric methyl α-galactopyranosides containing both *p*-bromobenzoate (λ_{max} 245 nm) and *p*-methoxycinnamate (λ_{max} 311 nm) were prepared. This set is comprised of six dibenzoate dicinnamates, four monocinnamate tribenzoates, and four monobenzoate tricinnamates. Each derivative provided a unique c.d. spectrum, distinct from the spectra of all others, including

TABLE I

C.D. DATA FOR "HOMO" INTERACTIONS. THE SIX DIBENZOATE DIACETATES AND THE SIX DICINNAMATE DIACETATES OF THE BASIS SET

Entry	*Compound*[a]	λ^b ($\Delta\varepsilon$)	λ ($\Delta\varepsilon$)	$\Delta\varepsilon = 0^c$	A^d
1	BAAB	236 (+2)	253 (−3)	243	−5
2	BBAA	236 (−21)	253 (+52)	242	+73
3	ABAB	232 (+4)	251 (−13)	239	−17
4	BABA	234 (−4)	251 (+28)	239	+32
5	ABBA	234 (−9)	251 (+39)	240	+48
6	AABB	236 (+9)	251 (−14)	243	−23
7	CACA	287 (−15)	321 (+34)	304	+49
8	AACC	286 (+13)	322 (−19)	306	−32
9	ACAC	281 (+9)	321 (−15)	304	−24
10	CAAC	286 (+3)	320 (−4)	305	−7
11	ACCA	286 (−19)	322 (+40)	304	+59
12	CCAA	286 (−38)	322 (+63)	306	+101

[a]Derivatization of the four positions is represented in the order 2,3,4,6, by A = acetate, B = *p*-bromobenzoate, and C = *p*-methoxycinnamate. [b]Extrema, in nm. [c]Point where $\Delta\varepsilon$ changes sign, in nm. [d]A value (difference between extrema).

TABLE II

C.D. DATA FOR "HETERO" INTERACTIONS. THE TWELVE MONOBENZOATE MONOCINNAMATE DIACETATES OF THE BASIS SET

Entry	*Compound*[a]	λ^b ($\Delta\varepsilon$)	λ ($\Delta\varepsilon$)	$\Delta\varepsilon = 0^c$
1	AACB	246 (+4)	286 (−2)	260
2	AABC	248 (+7)	306 (−5)	262
3	BACA	248 (−8)	308 (+10)	261
4	CABA	252 (−1)	295 (+6)	258
5	ACAB	249 (+3)	307 (−3)	259
6	ABAC	248 (+4)	305 (−4)	262
7	CAAB	243 (+1)	304 (+1)	
8	BAAC	243 (+3)	297 (−2)	259
9	CBAA	246 (−14)	308 (+12)	260
10	BCAA	248 (−13)	308 (+13)	261
11	ACBA	252 (−3)	310 (+9)	258
12	ABCA	244 (−15)	308 (+14)	260

[a]Derivatization of the four positions is represented, in the order 2,3,4,6, by A = acetate, B = *p*-bromobenzoate, and C = *p*-methoxycinnamate. [b]Extrema in nm. [c]Point where $\Delta\varepsilon$ changes sign, in nm.

those of the analogous glucoside derivatives previously reported[6].

To gain further insight into the interactions resulting in these complex spectra, all 24 permutational isomers of the galactoside containing only two of the exciton chromophores and two "nonchromophoric" acetates (to mimic conformations in tetrachromophoric species[6]) were also prepared. The c.d. spectra of these derivatives thus constituted a basis set of all of the possible pairwise interactions in tetrachromophoric derivatives. This set includes six diacetate dibenzoates and six diacetate dicinnamates (see Table I), as well as twelve diacetate monobenzoate monocinnamates (see Table II). Summation of the six appropriate basis-set spectra representing the contributing pairwise interactions in a tetrachromophoric derivative thus provides an empirical calculation for the c.d. spectrum of this derivative.

For example, Fig. 1a–f shows the six basis-set spectra contributing to the spectrum of the 2,3-dibenzoate 4,6-dicinnamate, which can be represented by BBCC; namely, derivatization of the four positions is represented, in the order 2, 3, 4, 6, by A = acetate, B = *p*-bromobenzoate, and C = *p*-methoxycinnamate. The first four (a–d) are nondegenerate ("hetero") B–C interactions with extrema at ~246 and 305 nm. The first two (a,b) are much smaller (expanded relative to all others, which are on the same scale), a pattern true for all 2,6 and 3,6 interactions due to the greater interchromophoric distance. (The magnitude of coupling has been shown[7] to be inversely proportional to the square of the interchromophoric distance.) The contributing 4,6 C–C interaction (e) and the 2,3 B–B interaction (f) follow. These degenerate ("homo") interactions have extrema centered about the λ_{max} of each chromophore, and are larger than the corresponding nondegenerate B–C interactions. Finally, the sum of all six interactions shown in Fig. 1g represents an empirical calculation of the c.d. spectrum of the tetra-

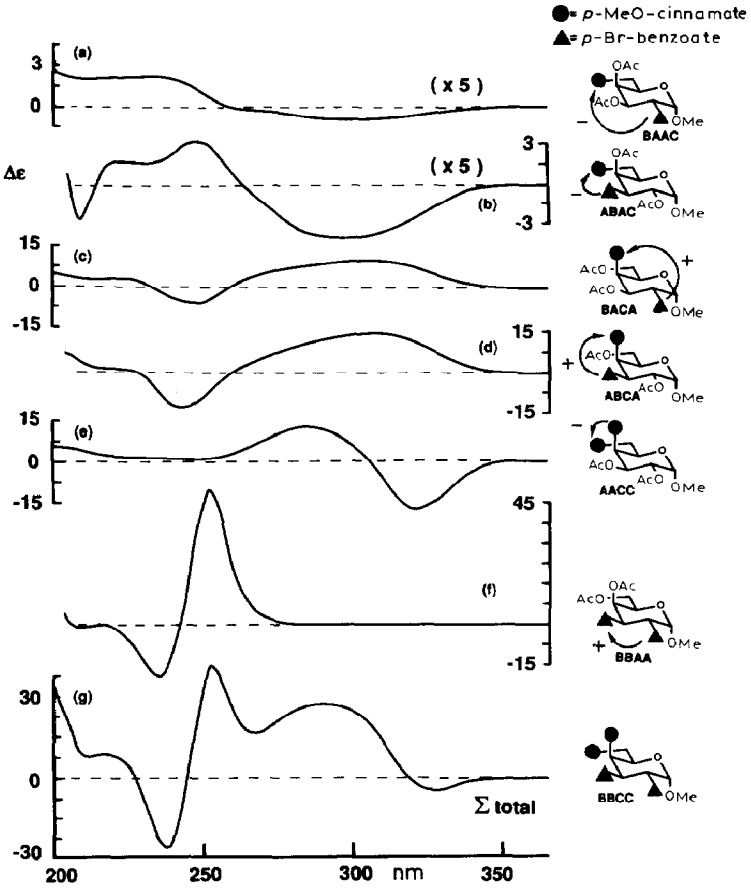


Fig. 1. Calculation of the c.d. curve of methyl α-D-galactopyranoside 2,3-dibromobenzoate 4,6-dimethoxycinnamate BBCC. (a–d) The c.d. curves of the four contributing hetero interactions. (e) C.d. spectrum of AACC, representing the 4,6 C/C "homo" interaction. (f) C.d. spectrum of BBAA representing the 2,3 B/B homo interaction. (g) Summation of the six contributing interactions gives the empirically calculated c.d. spectrum (Σ Total) of the 2,3-dibenzoate 4,6-dicinnamate. (For additional examples of this type of calculation, see ref. 6).

chromophoric BBCC. This curve clearly reflects the large, positive, B–B interaction, a negative C–C interaction, and a net positive B–C interaction.

The excellent agreement between calculated and observed spectra for this (see Fig. 2a, BBCC) and the other five dibenzoate dicinnamates is shown in Fig. 2. Calculations are also excellent for the four monocinnamate tribenzoates (see Fig. 3) and the four monobenzoate tricinnamates (see Fig. 4) as well. (Numerical data comparing calculated *versus* observed spectra are given in Table III.) In the latter two groups, calculated spectra are the sum of three homo and three hetero interactions. The accuracy of these calculations thus confirms the pairwise additivity of the interchromophoric interactions.

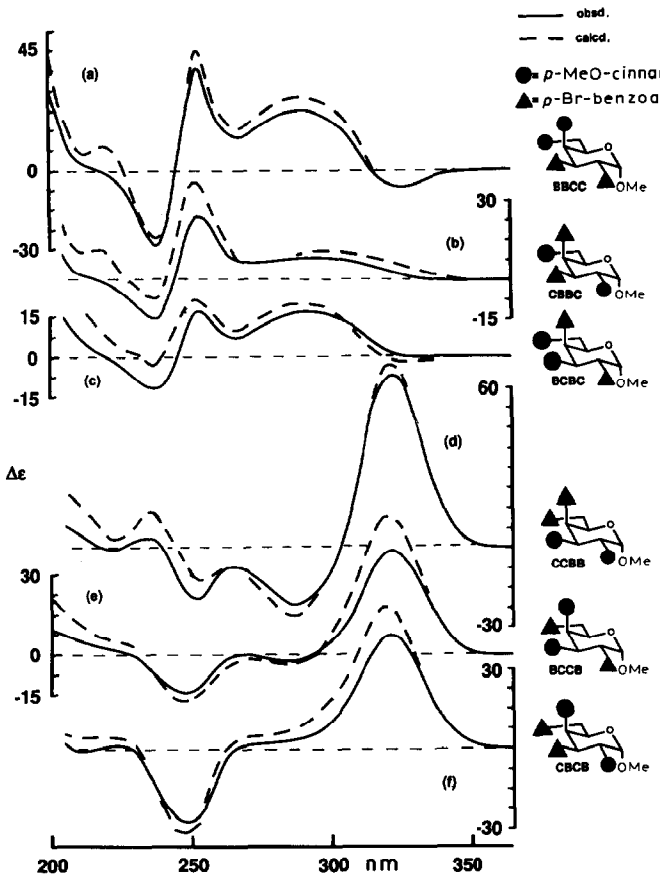


Fig. 2. Comparison of calculated and observed c.d. spectra for the six dibenzoate dicinnamates of methyl α-D-galactopyranoside.

DISCUSSION

The important practical consequence of understanding pairwise contributions to exciton-coupled c.d. spectra is twofold: (*1*) c.d. spectra of multichromophoric derivatives are predictable given knowledge of the magnitudes and signs of the various types of contributing interactions; and (*2*) c.d. curves of unknown derivatives are easily interpretable because they suggest the types, magnitudes, and signs of the dominant contributing pairwise interactions.

When interpreting these complex spectra, we noted that homo interactions are stronger than the others, and most easily recognized. Furthermore, in tetrachromophoric hexopyranoside derivatives, the vicinal 2,3 and 3,4 interactions dominate the c.d. curves, owing to their short interchromophoric distances. In glucosides, these interactions are of opposite sign; in galactosides, both are positive; and in mannosides, both are negative.

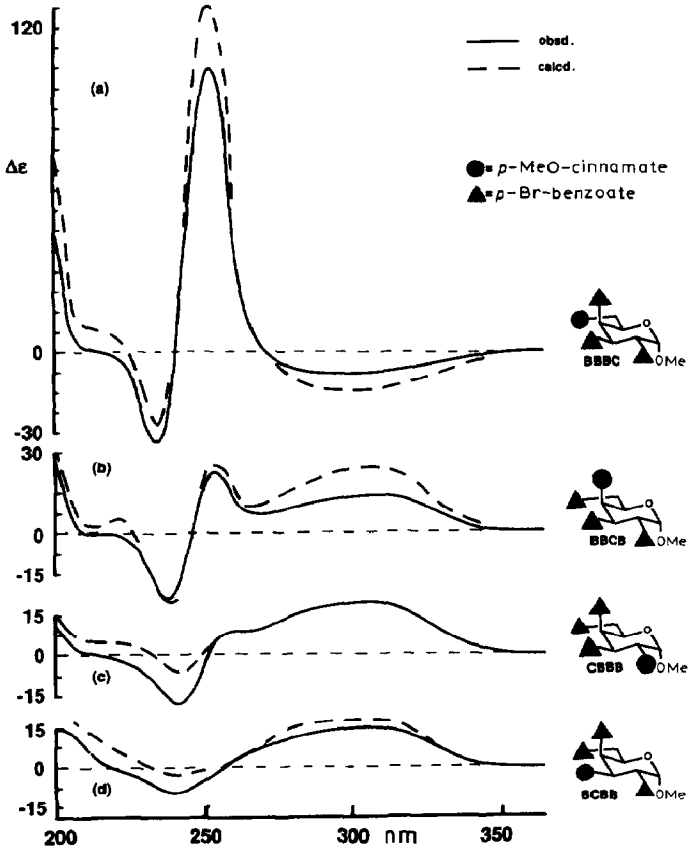


Fig. 3. Comparison of calculated and observed c.d. spectra for the four monocinnamate tribenzoates.

On inspecting the spectrum of CBBC (see Fig. 2b), we detect a large positive B–B and a smaller positive B–C interaction. This spectrum must belong to a galactoside derivative which satisfies these requirements, narrowing the assignment to either BBCC or CBBC. The absence of any detectable C–C interaction in the 311-nm range favors assignment to CBBC. (The presence of a negative C–C interaction in the spectrum of BBCC may be seen in Fig. 2a). The spectra representing the two major contributing interactions are shown in Fig. 5a,b. The sum of just these two contributions compares well with the observed CBBC spectrum (see Fig. 5c). Similarly, the spectrum of CBCC (see Fig. 4a) is dominated by a large positive B–C coupling. Two positive B–C interactions (see Fig. 6a,b) account for all but a small, positive C–C component of the spectrum, as seen in Fig. 6c.

We are developing an oligosaccharide derivatization sequence which results in easily identifiable tetrachromophoric methyl glycosides[8]. This sequence, shown in Fig. 7, is similar to a conventional sequence of methylation, methanolysis, and acetylation to provide partially methylated and acetylated methyl glycosides[9]. Here we replace the methyls which tag the free hydroxyls with one chromophore, and

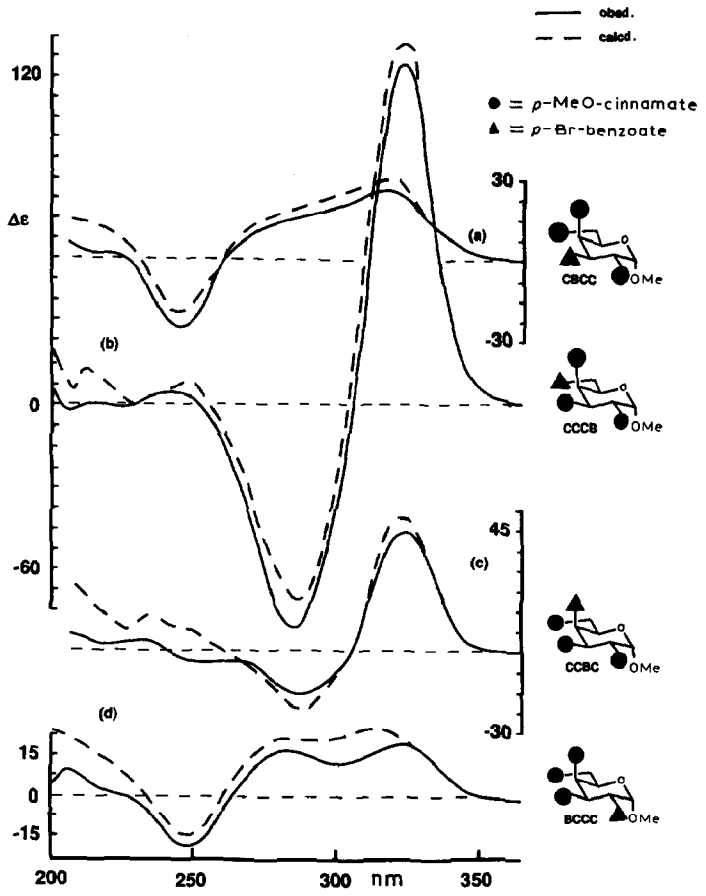


Fig. 4. Comparison of calculated and observed c.d. spectra for the four monobenzoate tricinnamates.

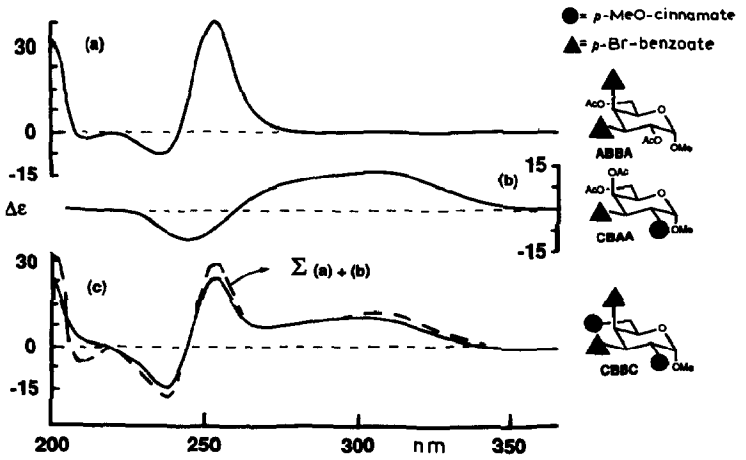


Fig. 5. Calculation of the c.d. spectrum of CBBC by addition of only the two dominant contributing interactions. (a) The major 3,4 homo interaction. (b) The major 2,3 hetero interaction. (c) The sum of these two major interactions [Σ(a) + (b)] compared to the observed c.d. spectrum of CBBC.

TABLE III

C.D. DATA FOR TETRACHROMOPHORIC DERIVATIVES

Entry	*Compound*[a]		λ[b] ($\Delta\varepsilon$)	λ ($\Delta\varepsilon$)	λ ($\Delta\varepsilon$)	λ ($\Delta\varepsilon$)	$\Delta\varepsilon = 0$[c]
25	CBCB	obs.	248 (−29)	321 (+44)			263
		calc.	249 (−33)	318 (+57)			261
26	BCBC	obs.	237 (−13)	252 (+19)	288 (+17)		244
		calc.	239 (−2)	251 (+24)	289 (+21)		240
27	BCCB	obs.	248 (−18)	287 (−3)	321 (+41)		267, 294
		calc.	248 (−20)	286 (−4)	320 (+53)		267, 293
28	CBBC	obs.	237 (−16)	253 (+26)	301 (+11)		244
		calc.	239 (−10)	252 (+41)	302 (+12)		241
29	BBCC	obs.	238 (−30)	253 (+37)	290 (+23)	325 (−8)	246, 316
		calc.	239 (−29)	253 (+48)	290 (+30)	328 (−8)	245, 319
30	CCBB	obs.	236 (+5)	252 (−22)	288 (−26)	321 (+67)	241, 303
		calc.	236 (+16)	252 (−15)	287 (−28)	321 (+71)	244, 303
31	BCCC	obs.	249 (−21)	283 (+18)	322 (+21)		261
		calc.	249 (−18)	283 (+23)	308 (+27)		259
32	CBCC	obs.	245 (−27)	317 (+28)			259
		calc.	245 (−21)	318 (+33)			259
33	CCBC	obs.	236 (+6)	287 (−19)	325 (+47)		239, 305
		calc.	236 (+15)	287 (−25)	324 (+52)		258, 305
34	CCCB	obs.	243 (+6)	286 (−85)	322 (+127)		253, 304
		calc.	247 (+10)	287 (−72)	323 (+130)		256, 303
35	BBBC	obs.	235 (−34)	252 (+105)	298 (−7)		240, 274
		calc.	235 (−27)	252 (+128)	306 (−13)		241, 274
36	BBCB	obs.	239 (−25)	254 (+27)	310 (+14)		245
		calc.	239 (−29)	254 (+28)	308 (+26)		246
37	BCBB	obs.	239 (−10)	308 (+16)			257
		calc.	240 (−3)	308 (+21)			256
38	CBBB	obs.	241 (−20)	308 (+20)			251
		calc.	240 (−10)	310 (+20)			249
82	BBCB		238 (−27)	253 (+29)	311 (+15)		245
83	BCBB		247 (−9)	309 (+19)			257
84	CBBB		243 (−15)	309 (+18)			254
85	CBCB		249 (−31)	321 (+38)			263

[a]Derivatization of the four positions is represented in the order 2,3,4,6, by B = *p*-bromobenzoate and C = *p*-methoxycinnamate. [b]Extrema, in nm. [c]Point where $\Delta\varepsilon$ changes sign, in nm.

the acetates which tag the linkage positions with the other chromophore. The resulting tetrachromophoric residues can be separated by high-performance liquid chromatography (h.p.l.c.). The ratio of the two chromophores is determined by ultraviolet (u.v.) spectroscopy without recourse to mass spectrometry, and finally, the c.d. spectra reveal the identity of the sugar, the linkage patterns, and even the D or L configuration. It is important to note that all spectra recorded here were performed on 20 nmol of material, and yet we have found that identification is possible with much less.

Methanolysis of the perbenzylated oligosaccharide (see Fig. 7, Step 2) yields both anomers of the methyl glycoside; this ultimately results in two tetrachromophoric glycosides for each residue. We have prepared four tetrachromophoric

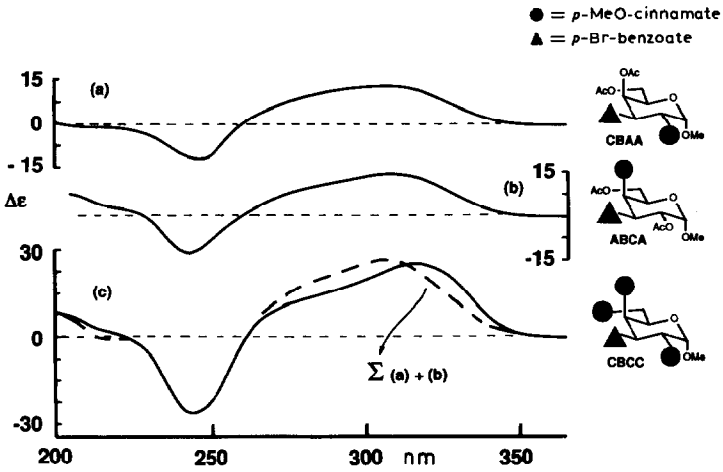


Fig. 6. Calculation of the c.d. spectrum of CBCC by addition of only the two dominant contributing interactions. (a) The large 2,3 hetero interaction. (b) The large 3,4 hetero interaction. (c) The sum of these two major interactions [Σ(α) + (b)] compared to the observed c.d. spectrum of CBCC.

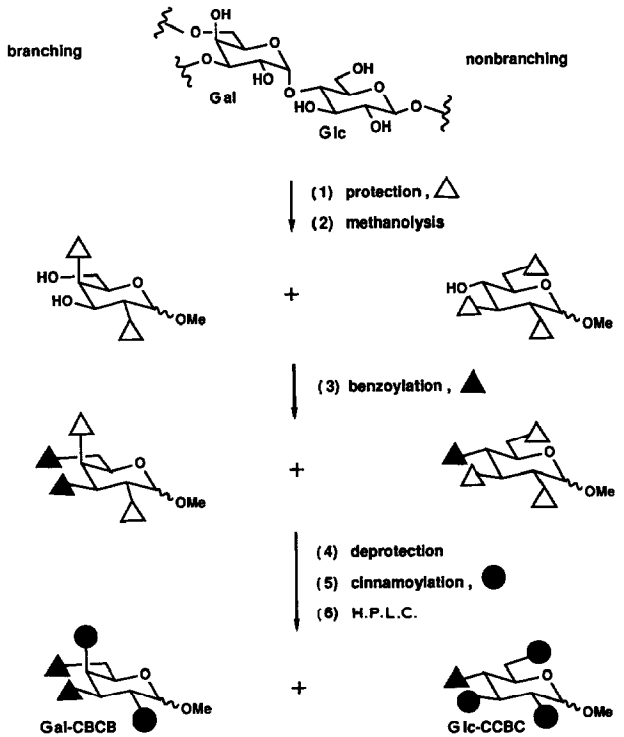


Fig. 7. Oligosaccharide derivatization sequence of a hypothetical disaccharide subunit to provide tetrachromophoric D-hexopyranoside derivatives for c.d. identification. The 3,6-branched D-galactoside residue gives both anomers of the dibenzoate dicinnamate Gal-CBCB, while the 4-linked D-glucoside residue gives both anomers of the monobenzoate tricinnamate Glc-CCBC.

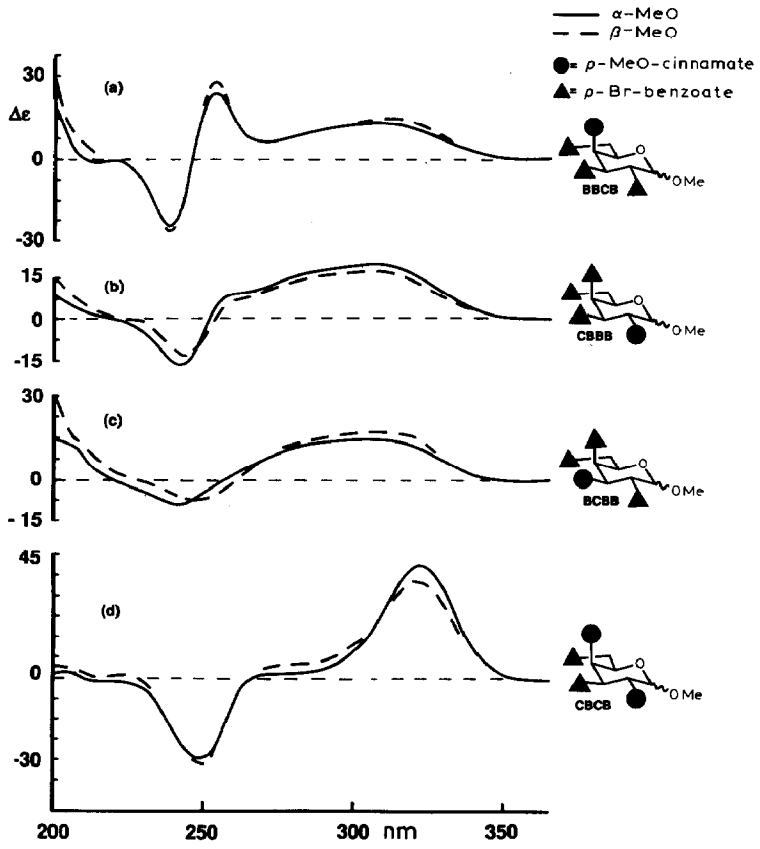


Fig. 8. Comparison of the c.d. spectra of *α versus β* anomers of similarly substituted tetrachromophoric methyl D-galactopyranosides, demonstrating the small effect of the orientation of the methyl group on the spectra.

methyl β-galactosides, and their c.d. spectra are compared to the corresponding methyl α-glycoside in Fig. 8. The orientation of the anomeric methyl group causes only slight changes in the c.d. spectra. Thus, the derivatization sequence provides *two* identifiable tetrachromophoric species for each residue in the oligosaccharide, thus ensuring the accuracy of the assignments. Whereas g.l.c. retention-times are crucial for assignment in conventional methylation analysis, in this approach the h.p.l.c. retention times merely provide yet another check on the system.

We have described the spectroscopic basis for a novel oligosaccharide micro-analysis which simultaneously provides identification of the sugar residues, their linkage patterns, and their absolute configurations.

EXPERIMENTAL[10]

General. — Preparation, purification by preparative t.l.c. and h.p.l.c., characterization by ^{1}H-n.m.r. spectroscopy (see Table IV), and u.v./c.d. spectros-

copy of the methyl D-galactopyranoside derivatives, as well as empirical calculation of c.d. spectra by computer summation of the appropriate, normalized (to 10μM) basis-set spectra, were all performed as previously described[6] for the analogous series of methyl α-D-glucopyranoside derivatives.

All compounds were prepared by appropriate sequences of partial acetylation, (*p*-bromobenzoyl)ation and (*p*-methoxycinnamoyl)ation of the parent methyl α-D-galactopyranoside. Sequences followed the typical reaction-order for esterification of the four hydroxyl groups, namely, 6 > 2 = 3 > 4. For example, partial (*p*-methoxycinnamoyl)ation of the methyl galactoside with 2 equiv. of *p*-methoxycinnamoyl chloride in pyridine with 4-(dimethylamino)pyridine (DMAP) as the catalyst at 60° afforded the following eleven *p*-methoxycinnamate derivatives: 2,3,4,6-tetracinnamate, 2,3,6- and 2,4,6-tricinnamates, 2,3-, 2,4-, 2,6-, 3,6-, and 4,6-dicinnamates, and the 2-, 3-, and 6-monocinnamates (see Scheme 2).

A strategy for separation of these eleven products was developed on the basis of 2D-t.l.c. with 7:3 EtOAc–hexane and 1:24 MeOH–CH_2Cl_2 as shown in Fig. 9. Separation was accomplished first by a 0–10% gradient of MeOH–CH_2Cl_2 on silica gel, followed by further purification on silica gel with EtOAc–hexane. MeOH–CH_2Cl_2 distinguishes on the basis of the number of free hydroxyl groups whereas EtOAc–hexane discriminates better between the various substitution patterns. In a similar fashion, partial (*p*-bromobenzoyl)ation afforded the following eight *p*-bromobenzoates derivatives: 2,3,6- and 2,4,6-tri-, 4,6-, 2,6-, and 3,6-di-, and 2-, 3-, and 6-mono- (see Scheme 1). Each of these products was then further derivatized at the remaining free hydroxyl groups with acetate or *p*-methoxycinnamate substituents, or both. Partial cinnamoylation of the three monobenzoates with 1.5 equiv. of *p*-methoxycinnamoyl chloride afforded three compounds each, and these were all further derivatized to tetra-*O*-acyl derivatives, as shown in Scheme 2.

Partial acetylation of methyl α-D-galactopyranoside with 2 equiv. of acetic anhydride in pyridine containing DMAP afforded the 3,6-diacetate as the major product, along with the 2,6-diacetate and the 4,6-diacetate (see Scheme 3). These were separated by flash chromatography with 1:4 EtOAc–hexane. The 4,6-derivatives were obtained from the 2,3-diacetates, which were conveniently prepared by acetylation of the 4,6-benzylidene acetals followed by deprotection with 1% of TsOH in MeOH, for 6 h at 0°. These diacetates were further derivatized by benzoylation or cinnamoylation or both. In three different cases, 3→4 acetyl migration was observed when either benzoylating or cinnamoylating the final free 4-hydroxyl group, as indicated in Scheme 3. These migration products had been prepared by an independent route, thus facilitating their identification. In no case were benzoyl or cinnamoyl migrations observed. This 3→4 migration proceeds *via* an ortho acid type of intermediate which forms a *cis*-fused, five-membered ring.

Although partial (*p*-bromobenzoyl)ations and (*p*-methoxycinnamoyl)ations were performed with the conventional acid chlorides in pyridine with DMAP as the catalyst, modification of the benzoylation procedure of Brown and Koreeda[11], which uses benzoic triflic anhydride, provided alternative methods for per-

TABLE IV

^{1}H-N.M.R. DATA

Compound	*αOMe*	*H1 (d)*	*H2 (dd)*	*H3 (dd)*	*H4 (d or dd)*	*H5 (dd or ddd)*	*H6 (d or dd's)*	*-OAc*
Basis set compounds								
1 BCAA	3.40	5.14 (3.6)	5.40 (3.6, 10.7)	5.67 (10.7, 3.4)	5.58 (3.4, 1.0)	4.28 (1.0, 6.3)	4.14 (6.3, 2H)	2.16 2.06
2 CBAA	3.45	5.08 (3.5)	5.47 (m)	5.66–5.60 (m, 2 H)		4.30 (6.5)	4.14 (6.5, 2H)	2.10 2.05
3 BACA	3.40	5.16 (3.6)	5.39 (3.6, 10.2)	5.60 (10.2, 3.4)	5.62 (3.4)	4.31–4.08 (m, 3H)		2.05 1.91
4 CABA	3.44	5.14 (3.6)	5.33 (3.6, 10.8)	5.54 (10.8, 3.3)	5.72 (3.3)	4.32 (6.2, 5.3)	4.19 (6.2, 11.2) 4.12 (5.3, 11.2)	2.01 1.91
5 BAAC	3.39	5.13 (3.6)	5.33 (3.6, 10.3)	5.54 (10.3, 3.4)	5.56 (3.4)	4.32–4.16 (m, 3H)		2.17 1.92
6 CAAB	3.41	5.07 (3.6)	5.29 (3.6, 10.7)	5.48 (10.7, 3.4)	5.56 (3.4, 0.7)	4.35 (0.7, 6.2, 5.7)	4.45 (6.2, 9.9) 4.26 (5.7, 9.9)	2.16 1.96
7 ABCA	3.45	5.05 (3.6)	5.43 (3.6, 10.8)	5.60 (10.8, 3.4)	5.71 (3.4, 1.0)	4.31 (1.0, 6.7, 6.0)	4.21 (6.7, 11.1) 4.11 (6.0, 11.1)	2.04 2.01
8 ACBA	3.45	5.09 (3.6)	5.33 (3.6, 10.8)	5.57 (10.8, 3.3)	5.79 (3.3)	4.34 (6.3, 6.5)	4.17 (6.3, 11.2) 4.14 (6.5, 11.2)	2.06 2.01
9 ABAC	3.44	5.01 (3.6)	5.39 (3.6, 10.7)	5.55 (10.7, 3.3)	5.65 (3.3)	4.32–4.18 (m, 2H)		2.10 2.02
10 ACAB	3.41	5.02 (3.6)	5.29 (3.6, 10.8)	5.50 (10.8, 3.4)	5.63 (3.4)	4.36 (5.4, 6.6)	4.45 (6.6, 10.0) 4.24 (5.4, 10.0)	2.15 2.06
11 AABC	3.44	5.10 (3.6)	5.23 (3.6, 10.8)	5.46 (10.8, 3.3)	5.76 (3.3)	4.34 (m, 2H)	4.21 (5.2, 13.3)	2.08 1.93
12 AACB	3.41	5.06 (3.6)	5.24 (3.6, 10.8)	5.43 (10.8, 3.3)	5.67 (3.3)	4.35 (6.6, 5.7)	4.51 (6.6, 10.6) 4.25 (5.7, 10.6)	2.08 1.97
13 BBAA	3.43	5.14 (3.6)	5.51 (3.6, 10.8)	5.74 (10.8, 3.4)	5.63 (3.4, 1.2)	4.31 (1.2, 6.5, dt)	4.15 (6.5)	2.12 2.05
14 BABA	3.41	5.20 (3.6)	5.38 (3.6, 10.5)	5.65 (10.5, 3.2)	5.72 (3.2, 0.8)	4.33 (0.8, 6.5)	4.16 (m, 2H)	2.02 1.87
15 BAAB	3.38	5.13 (3.6)	5.34 (3.6, 10.0)	5.58 (10.0, 3.4)	5.57 (3.4)	4.36 (5.8, 6.3)	4.46 (6.3, 10.0) 4.27 (5.8, 10.0)	2.17 1.92

16	ABBA	3.47	5.09 (3.5)	5.42 (3.5, 10.8)	5.65 (10.8, 3.3)	5.82 (3.3)	4.36 (dd)	4.17 (m, 2H)	2.01 2.00
17	ABAB	3.43	5.02 (3.6)	5.40 (3.6, 10.7)	5.57 (10.7, 3.3)	5.68 (3.3, 0.7)	4.40 (0.7, 6.7, 5.2)	4.46 (6.7, 9.9) 4.26 (5.2, 9.9)	2.10 2.02
18	AABB	3.43	5.09 (3.6)	5.22 (3.6, 10.9)	5.46 (10.9, 3.4)	5.77 (3.4)	4.40 (5.4, 6.7)	4.49 (6.7, 10.4) 4.26 (5.4, 10.4)	2.08 1.93
19	CCAA	3.42	5.09 (3.5)	5.36 (3.5, 10.0)	5.56 (10.0, 3.4)	5.58 (3.4)	4.26 (6.6)	4.13 (6.6, 2H)	2.15 2.05
20	CAAC	3.42	5.08 (3.7)	5.29 (3.7, 10.5)	5.47 (10.5, 3.4)	5.53 (3.4)	4.30–4.20 (m, 3H)		2.17 1.96
21	CACA	3.42	5.11 (3.5)	5.34 (3.5, 10.8)	5.49 (10.8, 3.3)	5.62 (3.3)	4.27 (6.6, 5.7)	4.21 (6.6, 10.7) 4.10 (5.7, 10.7)	2.04 1.95
22	ACCA	3.44	5.06 (3.5)	5.34 (3.5, 10.8)	5.49 (10.8, 3.4)	5.68 (3.4)	4.26 (6.6, 5.9)	4.17 (6.6, 11.0) 4.11 (5.9, 11.0)	2.06 2.04
23	ACAC	3.43	5.02 (3.6)	5.29 (3.6, 10.8)	5.49 (10.8, 3.3)	5.61 (3.3)	4.30–4.17 (m, 3H)		2.15 2.06
24	AACC	3.42	5.06 (3.5)	5.24 (3.5, 10.9)	5.41 (10.9, 3.4)	5.65 (3.4)	4.31 (6.3, 4.0)	4.35 (6.3, 9.2) 4.19 (4.0, 9.2)	2.08 1.97
Additivity test cases—Tetrachromophoric derivatives									
25	CBCB	3.47	5.17 (3.5)	5.56 (3.5, 10.7)	5.75 (10.7, 3.4)	5.84 (3.4)	4.48 (5.4, 6.8)	4.57 (6.8, 10.5) 4.32 (5.4, 10.5)	
26	BCBC	3.45	5.26 (3.6)	5.50 (3.6, 10.8)	5.81 (10.8, 3.4)	5.89 (3.4)	4.45–4.35 (m, 2H)		
27	BCCB	3.42	5.23 (3.6)	5.50 (3.6, 10.1)	5.79 (10.1, 3.4)	5.81 (3.4)	4.47 (5.4, 6.8)	4.27 (5.6, 11.0) 4.56 (6.8, 10.6) 4.32 (5.4, 10.6)	
28	CBBC	3.49	5.19 (3.6)	5.56 (3.6, 10.7)	5.77 (10.7, 3.3)	5.92 (3.3)	4.47 (5.7, 6.4)	4.39 (6.4, 11.1) 4.27 (5.7, 11.1)	
29	BBCC	3.46	5.23 (3.6)	5.60 (3.6, 10.0)	5.82 (10.0, 3.4)	5.84 (3.4)	4.46–4.38 (m, 2H)	4.27 (8.2, 13.7)	
30	CCBB	3.46	5.20 (3.5)	5.47 (3.5, 10.8)	5.71 (10.8, 3.4)	5.91 (3.4)	4.55–4.46 (m, 2H)	4.31	
31	BCCC	3.43	5.23 (3.6)	5.50 (3.5, 10.4)	5.79–5.75 (m, 2H)	4.41–4.36 (m, 2H)	4.26 (8.2, 13.6)		
32	CBCC	3.48	5.17 (3.5)	5.56 (3.5, 10.6)	5.73 (10.6, 3.3)	5.81 (3.3)	4.45–4.37 (m, 2H)	4.25 (8.3, 13.6)	
33	CCBC	3.47	5.20 (3.6)	5.47 (3.6, 10.8)	5.70 (10.8, 3.3)	5.89 (3.3)	4.5–4.2 (m, 3H)		
34	CCCB	3.44	5.17 (3.5)	5.47 (3.5, 10.8)	5.67 (10.8, 3.4)	5.80 (3.4)	4.50 (5.4, 6.7)	4.55 (6.7, 10.6) 4.31 (5.4, 10.6)	
35	BBBC	3.47	5.27 (3.6)	5.59 (3.6, 10.2)	5.89 (10.2, 3.4)	5.92 (3.4)	4.51–4.38 (m, 2H)	4.29 (5.8, 11.1)	
36	BBCB	3.44	5.22 (3.6)	5.59 (3.6, 10.0)	5.87 (10.0, 3.5)	5.84 (3.5)	4.49 (5.4, 6.8)	4.58 (6.8, 10.5) 4.32 (5.4, 10.5)	

Table IV (continued)

Compound	*αOMe*	*H1 (d)*	*H2 (dd)*	*H3 (dd)*	*H4 (d or dd)*	*H5 (dd or ddd)*	*H6 (d or dd's)*	*-OAc*
37 BCBB	3.44	5.26 (3.6)	5.50 (3.6, 10.7)	5.82 (10.7, 3.4)	5.91 (3.4)	4.53 (m)	4.54 (6.8, 14.5) 4.33 (9.3, 14.5)	
38 CBBB	3.48	5.20 (3.5)	5.56 (3.5, 10.8)	5.79 (10.8, 3.4)	5.93 (3.4)	4.54 (m)	4.55 (6.8, 14.4) 4.33 (9.3, 14.4)	
p-*Methoxycinnamoylation products*								
39 CCCC	3.46	5.17 (3.5)	5.46 (3.5, 10.8)	5.65 (10.8, 3.4)	5.79 (3.4)	4.40–4.35 (m, 2H)	4.25 (8.2, 13.5)	
40 CCOC	3.43	5.10(2.8)	5.49	5.49	4.26	4.20 (6.1, 6.7)	4.55 (6.1, 11.4) 4.37 (6.7, 11.4)	
41 COCC	3.43	5.10 (3.6)	5.23 (3.6, 10.4)	4.38–4.25	5.62 (3.4)	4.38–4.25 (m, 4H)		
42 OCOC	3.46	4.90 (3.9)	4.15–4.05	5.19 (10.3, 3.1)	4.15–4.05 (m, 3H)		4.51 (6.2, 11.4) 4.33 (6.6, 11.4)	
43 COOC	3.41	5.00 (3.7)	5.14 (3.7, 10.0)	4.12–4.02 (m, 3H)			4.62 (6.3, 11.4) 4.31 (6.5, 11.4)	
44 COCO	3.43	5.03 (3.6)	5.27 (3.6, 10.4)	4.33 (10.4, 3.5)	5.47 (3.5)	4.07 (6.3, 7.9)	3.68 (6.3, 11.8) 3.51 (7.9, 11.8)	
45 CCOO	3.42	5.11 (1.6)	5.49	5.49	4.37	4.04–3.87 (m, 3H)		
46 OOCC	3.46	4.92 (2.8)	3.92 (2.8, 10.0)	4.03 (10.0, 3.3)	5.54 (3.2)	4.20 (6.8, 4.8)	4.35 (6.8, 10.9) 4.22 (4.8, 10.9)	
47 OCOO	3.46	4.91 (3.9)	4.13 (3.9, 10.3)	5.17 (10.3, 3.0)	4.24 (3.0)	3.98–3.86 (m, 3H)		
48 COOO	3.39	5.00 (3.7)	5.17 (3.7, 10.1)	4.09 (10.1, 3.4)	4.16 (3.4)	3.96–3.85 (m, 3H)		
49 OOOC	3.43	4.84 (3.3)	4.02–3.75 (m, 4H)				4.56 (6.2, 11.3) 4.29 (6.8, 11.3)	
p-*Bromobenzoylation products*								
50 BBOB	3.41	5.15 (3.3)	5.61 (3.3, 10.6)	5.68 (10.6, 2.8)	4.34–4.27 (m, 2H)		4.65 (6.1, 11.6) 4.51 (6.7, 11.6)	
51 OBOB	3.47	4.91 (3.9)	4.22–4.12	5.30 (10.3, 3.0)	4.22–4.12 (m, 3H)		4.61 (6.1, 11.4) 4.48 (6.6, 11.4)	
52 BOOB	3.36	5.01 (3.7)	5.24 (3.7, 10.1)	4.19–4.08 (m, 3H)			4.64 (5.8, 11.5) 4.48 (7.0, 11.5)	
53 BBOO	3.41	5.16	5.63	5.63	4.45	4.01–3.92 (m, 3H)		
54 OOBB	3.45	4.94 (3.8)	3.92 (3.8, 9.8)	4.06 (9.8, 3.2)	5.68 (3.2)	4.30 (m, 2H)	4.48 (8.9, 12.8)	
55 BOOO	3.38	5.04 (3.7)	5.24 (3.7, 9.6)	4.17–3.86 (m, 5H)				

56	OBOO	3.47	4.93 (3.9)	4.20 (3.9, 10.3)	5.25 (10.3, 3.0)	3.98–3.68 (m, 3H)			
57	OOOB	3.40	4.83 (3.3)	4.06–3.79 (m, 4H)				4.62 (5.7, 11.4) 4.47 (7.0, 11.4)	
Intermediate and other derivatives									
58	BCOO	3.39	5.17 (3.4)	5.52 (3.4, 10.7)	5.59 (10.7, 2.7)	4.37 (2.7)	4.02–3.91 (m, 3H)		
59	CBOO	3.44	5.10 (3.4)	5.52	5.62	4.44	4.00–3.91 (m, 3H)		
60	OCOB	3.45	4.90 (d)	4.18–4.05	5.21	5.18–4.05 (m, 3H)		4.61 (dd) 4.50 (dd)	
61	OBOC	3.48	4.91 (d)	4.18–4.09	5.28 (10.3, 3.1)	4.18–4.09 (m, 3H)		4.54 (6.6, 11.4) 4.31 (6.3, 11.4)	
62	COOB	3.39	5.00 (3.7)	5.14 (3.7, 9.8)	4.16–4.08 (m, 3H)			4.66 (5.7, 11.5) 4.50 (7.0, 11.5)	
63	BOOC	3.38	5.02 (3.7)	5.23 (3.7, 10.1)	4.15 (10.1, 3.5)	4.04 (3.5)	4.07 (6.5, 6.6)	4.62 (6.6, 11.5) 4.30 (6.5, 11.5)	
64	ABOA	3.42	4.97 (3.1)	5.44 (m, 3H)			4.10 (6.1, 6.6)	4.35 (6.1, 11.4) 4.24 (6.6, 11.4)	2.07 2.00
65	BAOA	3.37	5.09 (3.6)	5.37 (3.6, 10.7)	5.49 (10.7, 3.1)	4.12 (3.1)	4.07 (6.0, 6.6)	4.36 (6.0, 11.5) 4.25 (6.6, 11.5)	2.08 2.02
66	AAOB	3.38	4.98 (3.3)	5.24 (3.3, 10.8)	5.31 (10.8, 2.6)	4.18–4.12	4.18–4.12 (m, 2H)	4.58 (5.8, 11.6) 4.47 (6.9, 11.6)	2.09 2.07
67	ACOA	3.41	4.98 (2.9)	5.34 (2.9, 10.8)	5.40 (10.8, 2.8)	4.18 (2.8)	4.08 (6.1, 6.6)	4.35 (6.1, 11.5) 4.26 (6.6, 11.5)	2.08 2.05
68	AOCA	3.41	5.00 (3.7)	5.08 (3.7, 10.8)	4.28–4.10	5.51 (3.5)	4.07 (dd)	4.28–4.10 (m, 3H)	2.14 2.05
69	OCAA	3.47	4.90 (3.8)	4.00 (3.8, 10.7)	5.24 (10.7, 3.3)	5.48 (3.3)	4.16 (6.1, 6.8)	4.08 (m, 2H)	2.14 2.05
70	CAOA	3.40	5.04 (3.3)	5.33 (3.3, 10.6)	5.39 (10.6, 3.8)	4.13 (bs)	4.05 (6.1, 6.5)	4.35 (6.1, 11.5) 4.26 (6.5, 11.5)	
71	AAOC	3.40	4.98 (3.1)	5.24 (3.1, 10.7)	5.30 (10.7, 2.6)	4.13 (2.6)	4.08 (6.3, 6.6)	4.51 (6.3, 11.5) 4.30 (6.6, 11.5)	2.09 2.07
72	ACAA	3.42	5.00 (3.6)	5.26 (3.6, 10.8)	5.46 (10.8, 3.3)	5.55 (3.3, 0.9)	4.23 (0.9, 6.1)	4.11 (6.1, d, 2H)	2.13 2.06 2.05
73	CCOB	3.43	5.12	5.50 (m, 2H)		4.38–4.22 (m, 2H)		4.65 (dd) 4.53 (dd)	

Table IV (continued)

Compound	*αOMe*	*H1 (d)*	*H2 (dd)*	*H3 (dd)*	*H4 (d or dd)*	*H5 (dd or ddd)*	*H6 (d or dd's)*	*-OAc*
74 BCOC	3.40	5.15 (3.5)	5.50 (3.5, 10.7)	5.63 (10.7, 3.0)	4.26 (3.0)	4.20 (6.2, 6.6)	4.56 (6.2, 11.5)	
							4.37 (6.6, 11.5)	
75 CBOC	3.45	5.08 (3.1)	5.60 (m, 2H)		4.32	4.21 (6.3, 6.5)	4.56 (6.3, 11.5)	
							4.35 (6.5, 11.5)	
76 CBOB	3.44	5.08 (2.6)	5.58 (m, 2H)		4.33	4.29 (5.9, 6.7)	4.63 (5.9, 11.6)	
							4.51 (6.7, 11.6)	
77 OAOA	3.43	4.83 (3.9)	3.99–3.94	5.04 (10.3, 3.1)	3.99–3.94 (m, 3H)		4.31 (6.1, 11.4)	2.14
							4.21 (6.6, 11.4)	2.06
78 AOOA	3.37	4.89 (3.7)	5.00 (3.7, 9.7)	4.01–3.92 (m, 3H)			4.39 (5.9, 11.5)	2.13
							4.20 (6.8, 11.5)	2.08
79 AAOO	3.37	4.98 (2.5)	5.25 (m, 2H)		4.24	4.92–4.83 (m, 3H)		2.08
								2.06
80 AAOA	3.38	4.95 (2.9)	5.21 (2.9, 10.8)	5.27 (10.8)	4.09 (0.7)	4.01 (0.7, 6.0, 6.6)	4.32 (6.0, 11.4)	2.08
							4.21 (6.6, 11.4)	2.06
81 OOAA	3.42	4.82 (3.7)	3.78 (3.7, 9.9)	3.93 (9.9, 3.3)	5.31 (3.3)	4.08 (m, 3H)		2.12
								2.04
Methyl β-D-galactopyranoside derivatives								
82 βBBCB	3.56	4.68 (7.9)	5.70 (7.9, 10.5)	5.48 (10.5, 3.4)	5.81 (3.4, 0.7)	4.23 (0.7, 6.5, 6.8)	4.66 (6.5, 11.2)	
							4.36 (6.8, 11.2)	
83 βBCBB	3.55	4.67 (7.9)	5.61 (7.9, 10.5)	5.46 (10.5, 3.4)	5.86 (3.4, 0.7)	4.24 (0.7, 6.3, 6.8)	4.63 (6.3, 11.1)	
							4.34 (6.8, 11.1)	
84 βCBBB	3.59	4.65 (7.8)	5.59 (7.8, 10.3)	5.42 (10.3, 3.4)	5.88 (3.4)	4.25 (6.5, 6.9)	4.63 (6.5, 11.0)	
							4.35 (6.9, 11.0)	
85 βCBCB	3.57	4.61 (7.9)	5.62 (7.9, 10.4)	5.39 (10.4, 3.4)	5.80 (3.4, 0.5)	4.21 (0.5, 6.6, 6.7)	4.64 (6.6, 11.1)	
							4.35 (6.7, 11.1)	
86 βBBOB	3.51	4.61 (7.9)	5.68 (7.9, 10.3)	5.29 (10.3, 3.2)	4.30 (3.2)	4.03 (6.4, 7.2)	4.67 (7.2, 10.4)	
							4.56 (6.4, 10.4)	
87 βBOBB	3.54	4.59 (7.9)	5.29 (7.9, 10.0)	4.14–4.08	5.71 (2.9)	4.14–4.08 (2H)	4.56 (6.7, 11.4)	
							4.36 (6.4, 11.4)	
88 βOBBB	3.64	4.44 (7.8)	4.03 (7.8, 10.2)	5.33 (10.2, 3.6)	5.82 (3.6, 0.9)	4.20 (6.3, 6.9, 0.9)	4.61 (6.3, 11.0)	
							4.31 (6.9, 11.0)	
89 βOBOB	3.58	4.33 (7.7)	4.00 (7.7, 10.2)	5.10 (10.2, 3.3)	4.19 (3.3)	3.94 (6.4, 6.8)	4.63 (6.8, 11.4)	
							4.52 (6.4, 11.4)	

[a]Derivatization of the four positions is represented in the order 2,3,4,6, by A = acetate, B = *p*-bromobenzoate, C = *p*-methoxycinnamate, and O =

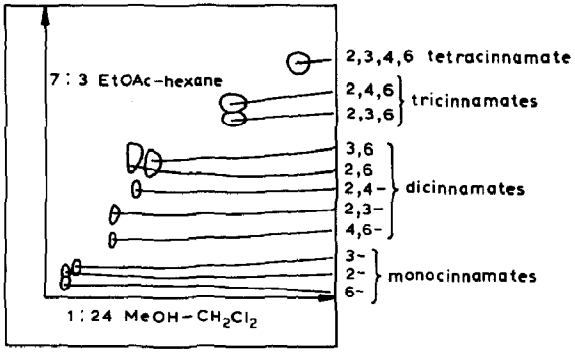
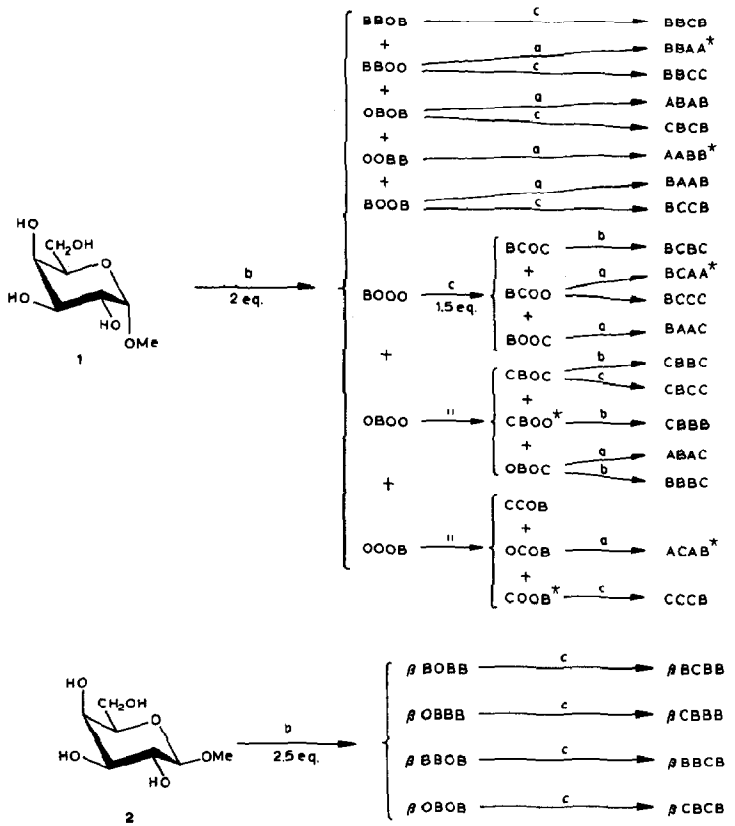


Fig. 9. 2D-T.l.c. of the crude reaction-mixture from the partial (*p*-methoxycinnamoyl)ation of methyl α-D-galactopyranoside.



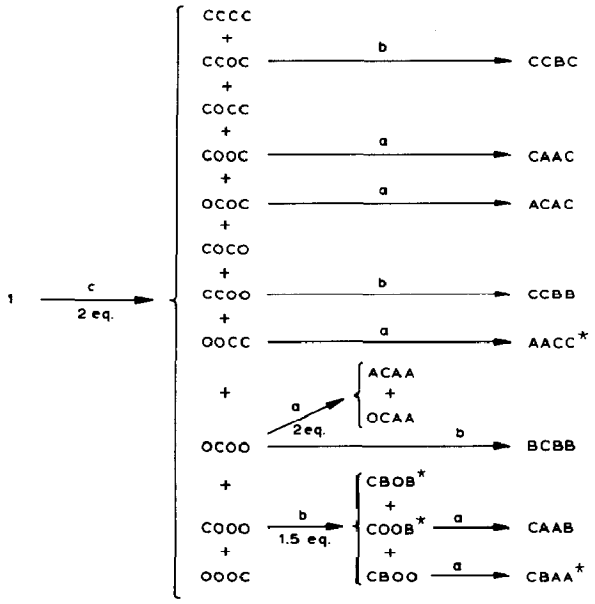
a = Ac_2O in excess (unless indicated otherwise)–pyridine–DMAP.

b = *p*-Bromobenzoyl chloride in excess (unless indicated otherwise)–pyridine–DMAP or benzoic triflic anhydride.

c = *p*-Methoxycinnamoyl chloride in excess (or as indicated)–pyridine–DMAP or cinnamic triflic anhydride.

*Prepared by an alternative route, as shown in Scheme 3.

Scheme 1. Partial (*p*-bromobenzoyl)ation products of both methyl α- and β-methyl D-galacto-pyranoside, and of derivatives obtained by further elaboration.



a = Ac_2O in excess (unless indicated otherwise)-pyridine-DMAP.

b = *p*-Bromobenzoyl chloride in excess (unless indicated otherwise)-pyridine-DMAP or benzoic triflic anhydride.

c = *p*-Methoxycinnamoyl chloride in excess (or as indicated)-pyridine-DMAP or cinnamic triflic anhydride.

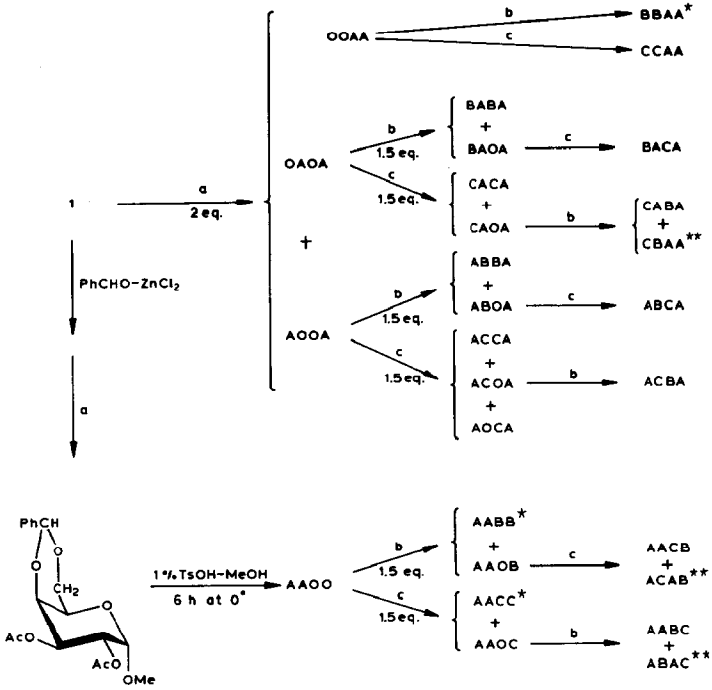
* Prepared by an alternative route, as shown in Scheme 1.

Scheme 2. Partial (*p*-methoxycinnamoyl)ation products of methyl α-D-galactopyranoside, and of derivatives obtained by further elaboration.

benzoylation and percinnamoylation. The mixed anhydrides were difficult to prepare, and yet preparation *in situ*[12] in the presence of pyridine (presumably leading to formation of the *N*-benzoylpyridinium triflate as the reactive benzoylating species[11]) afforded acylations which were efficient and quick.

General procedure. — To the sugar (1–5 mg) in dry dichloromethane (1 mL) and pyridine (200 μL) were added the acid chloride and silver triflate (3 equiv. of each per hydroxyl group) at room temperature under Ar. The reaction was usually complete within 1 h (t.l.c.). After quenching the reaction with an excess of methanol and filtration through Florisil with dichloromethane, the filtrate was evaporated under diminished pressure, and preparative t.l.c. gave the purified product in high yield (+90%).

All intermediates and final products were characterized by ^{1}H-n.m.r. spectroscopy (Bruker WM250 spectrometer operated at 250 MHz) for solutions in $CDCl_3$ with decoupling of ring protons whenever necessary to confirm a substitution pattern; the data are given in Table IV. U.v. measurements were performed with a Perkin–Elmer 320 u.v. spectrophotometer. C.d. spectra were recorded with a JASCO 500A spectropolarimeter driven by a JASCO DP500N data processor, four scans being taken from 200–400 nm. An IBM-PC operated with JASCO software was used to normalize all c.d. spectra to 10μM, as well as to perform all



a = Ac_2O in excess (unless indicated otherwise)-pyridine-DMAP.

b = *p*-Bromobenzoyl chloride in excess (unless indicated otherwise)-pyridine-DMAP or benzoic triflic anhydride.

c = *p*-Methoxycinnamoyl chloride in excess (or as indicated)-pyridine-DMAP or cinnamic triflic anhydride.

* Prepared by an alternative route, as shown in Scheme 1.

** Product of a 3→4 acetyl migration; prepared by another route.

Scheme 3. Preparation of methyl α-D-galactopyranoside diacetates, and subsequent derivatization to basis-set compounds.

TABLE V

H.P.L.C.[a] RETENTION TIMES IN MINUTES

BCAA 36	CBAA 31	BBAA 33	CCAA 57
BACA 30	CABA 29	BABA 17	CACA 50
BAAC 37	CAAB 20	BAAB 15	CAAC 59
ABCA 47	ACBA 37	ABBA 24	ACCA 65
ABAC 46	ACAB 26	ABAB 17	ACAC 48
AABC 33	AACB 31	AABB 21	AACC 58
CBCB 22	BBBC 16	BCCC 42	βCBCB 27
BCBC 24	CBBB 16	CBCC 39	βCBBB 20
BCCB 22	BCBB 14	CCBC 42	βBCBB 17
CBBC 27	BBCB 14	CCCB 42	βBBCB 17
BBCC 37			
CCBB 24			

[a]On YMC SiO_2 gel (5 μm) eluted with 3:7 EtOAc–hexane.

spectral summations for empirical calculations, which simply involved addition of the normalized c.d. spectra of the three or six appropriate dichromophoric derivatives corresponding to the constituent pairwise interactions of the tri- or tetrachromophoric derivatives, respectively.

Prior to measurement of c.d. spectra, all compounds were purified by h.p.l.c. with 3:7 EtOAc–hexane on YMC SiO_2 gel, 5μm at 2 mL/min (see Table V for retention times) to ensure the accuracy of determinations of concentration. Acetonitrile solutions were prepared which were 5–15μM; the concentrations were determined on the basis of the average *p*-methoxycinnamate u.v. ε values experimentally determined at 311 nm (where bromobenzoates are transparent): mono, ε_{mM} 24.00; di, 45.00; and tri, 68.00. In the case of the diacetate dibromobenzoates, the average dibromobenzoate value of ε_{mM} 38.20 at 245 nm was used.

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