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Configuration of Heptopyranoside and Heptofuranoside Side Chains: 2-Anthroate, a Powerful Chromophore for Exciton Coupled CD

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ABSTRACT The absolute configuration of the acyclic side chain of heptopyranosides and heptofuranosides was determined by exciton coupled CD, employing the strongly fluorescent 2-anthroate chromophore. The usage of this chromophore offers significant improvements over previous chemical and spectroscopic procedures since its intense fluorescence greatly facilitates the isolation and HPLC purification at the nanogram scale. The large amplitudes of the bisignate spectra allow CD manipulations in the 1×10^{-7} M range. *Chirality 9:699–712, 1997.* © 1997 Wiley-Liss, Inc.

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Heptopyranosides of the L-*glycero*-D-*manno*- and, more rarely, the D-*glycero*-D-*manno*-configurations, compounds **1** and **2,** respectively (Fig. 1), exist in the inner core of bacterial lipopolysaccharides of Gram-negative bacteria, usually in the form of di- or tri-heptosides.[1] It is suspected that heptofuranosides, such as L-*glycero*-D-*manno*-heptofuranose **3** might also constitute a portion of the bacterial lipopolysaccharide inner core.[2] The role of these compounds is under investigation and many synthetic studies aim to create reference libraries for comparisons with authentic samples as well as for immunological studies. It is expected that immunology will clarify the function of heptoses in the lipopolysaccharide system.

Heptopyranosides and heptofuranosides contain acyclic side chains with stereogenic centers, thus representing a challenge for assignment of absolute configurations. So far structural determination studies on this class of compounds have been limited to NMR, optical rotation, and melting point measurements of derivatives requiring, in most cases, large amounts of material.[3] As part of our ongoing efforts to develop new methodologies for assigning absolute configurations of acyclic systems using the exciton chirality circular dichroic method,[4] we have undertaken studies on heptopyranosides and heptofuranosides in order to devise a microscale approach for their structural elucidation.

The CD exciton chirality method[4] is a simple and versatile microscale method for determining the absolute configuration of chiral molecules containing two or more chromophores. A through space interaction of the electric transition moments of these chromophores gives rise to an exciton coupled "split" CD spectrum or "bisignate curve" whose amplitude is: (1) inversely proportional to the square of the interchromophoric distance; (2) proportional to the chromophoric absorption coefficient; and (3) dependent on the interchromophoric projection angle with a maximum at approximately 70° and minima at 0° or 180° (for 1,2-acylates).[4] In case of different chromophores, exciton coupling is still observed even when the absorption maxima of the chromophores are 100 nm apart. The sign of the exciton coupled split CD, reflecting the spatial arrangement of the chromophores, leads to assignment of the absolute configuration (or conformation) in a non-empirical manner, i.e., a positive bisignate CD is associated with a positive absolute twist between the electric transition moments of interacting chromophores, and vice versa. In rigid systems the assignment of absolute configurations is straightforward.

However, with acyclic systems such as acyclic polyols, the assignment of absolute configuration is more challenging due to possible equilibrium between several different conformers. Many methods (reference 5 and references therein) have been developed over the years, including a difference CD approach reported by Mori et al.[6] and a monochromophoric derivatization approach and exciton coupling between identical benzoate chromophores reported by Harada et al.[7] and Uzawa et al.[8] In the latter method, the absolute configurations of various diols have been assigned in an unambiguous manner. However, the CD spectra are sensitive to changes in the steric effects of substituents, and hence additional data regarding confor-

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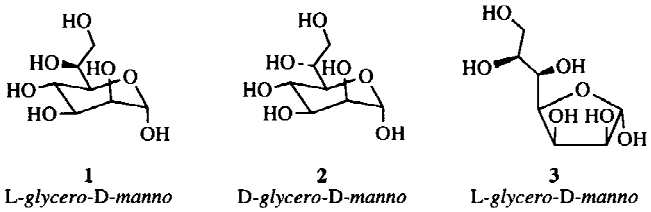


Fig. 1. Heptopyranosides **1** and **2** and heptofuranoside **3.**

mational distribution is usually required. This problem can be partially overcome by the bichromophoric exciton coupled CD approach.[9–11] In this method, two different chromophores are selectively introduced into a chiral molecule to provide "fingerprint" CD curves, which are used as references for assignments of absolute configurations of the respective structural moieties. This method has been successfully used for configurational assignments of various types of acyclic polyols and amino polyols.[9–11]

Nevertheless, configurational determinations of acyclic polyols may still be rather challenging and necessitate the employment of identical and different chromophores in a complimentary manner. Additionally, there is a constant need for new powerful chromophores to further enhance the sensitivity of UV, CD, and HPLC detection limits and facilitate microscale manipulations. In the following, we present a microscale method for the structural elucidation of the acyclic chain of heptopyranosides and heptofuranosides employing 2-anthroic acid as a highly fluorescent chromophore for derivatization of hydroxyl (and amino) groups; to the best of our knowledge this powerful chromophore has not been used for CD studies before.

MATERIALS AND METHODS

Derivatization Procedures

The chromophoric derivatization of hydroxyl compounds to the corresponding 2-anthroates was achieved either with 2-anthroylimidazole/1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) in MeCN or with 2-anthroic acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and dimethylaminopyridine (DMAP) in CH_2Cl_2. 9-Anthroylation was performed with 9-anthroyl fluoride in pyridine (see reference 27), 9-anthroic acid/EDC/DMAP in CH_2Cl_2, or 9-anthroyl tetrazole/DBU in MeCN. The derivatives were purified first by TLC followed by HPLC. The 2-anthroates are highly fluorescent, λ_{ex} 270 nm, λ_{em} 438 nm, with intense UV absorption at 258 nm, ε = 93,000 (Fig. 2, cyclohexyl 2-anthroate).[12] The ε value of cyclohexyl 2-anthroate was determined by averaging three exact weight measurements. 9-Anthroates are also highly fluorescent, λ_{ex} 362 nm, λ_{em} 456 nm, with a strong UV absorption at 251 nm, ε = 142,200[11] (Fig. 2, methyl 9-anthroate).

Synthesis of Acyclic Diols and Triols

The model D-*threo* acyclic triol (2R,3R)-1,2,3-pentanetriol **20b** (see Fig. 4) and D-*erythro* acyclic triol (2S,3R)-1,2,3-pentanetriol **23b** (see Fig. 5) were prepared by hydrazinolysis of pure D-xylose and D-arabinose, respectively, following literature procedures.[13]

Heptopyranosides were synthesized by addition of allyloxymethyl magnesium chloride to D-manno-aldehyde **6** (Scheme 1) followed by deprotection of the primary hydroxyl group by $(PPh_3)_3RhCl$ and Dabco to furnish a mixture of products.[14] In all compounds, there is a newly formed stereogenic center at C-6. The major compound **7** is expected by Cram's rule to have an L-*glycero*-D-*manno*-configuration, while anti-Cram product **8** should have the D-*glycero*-D-*manno* configuration. In addition to these compounds, an unexpected product **9** was formed; this is derived from inversion of starting aldehyde **6** at C-5, prior to the Grignard reaction.

The "C-5 inverted" compound **9** was assigned the L-*gulo* configuration based on NMR studies. An epimerization at C-5 results in an inversion of the chair form in order to accommodate the side chain in an equatorial position. This was shown experimentally by measuring the 1-H/2-H coupling constant. In compounds **7** and **8,** the hydrogens at C-1 and C-2 adopt an equatorial position and their coupling constant is small (~1.5 Hz), while in the inverted compound **9,** these hydrogens are axial giving rise to a $J_{1,2}$ of 8.1 Hz.

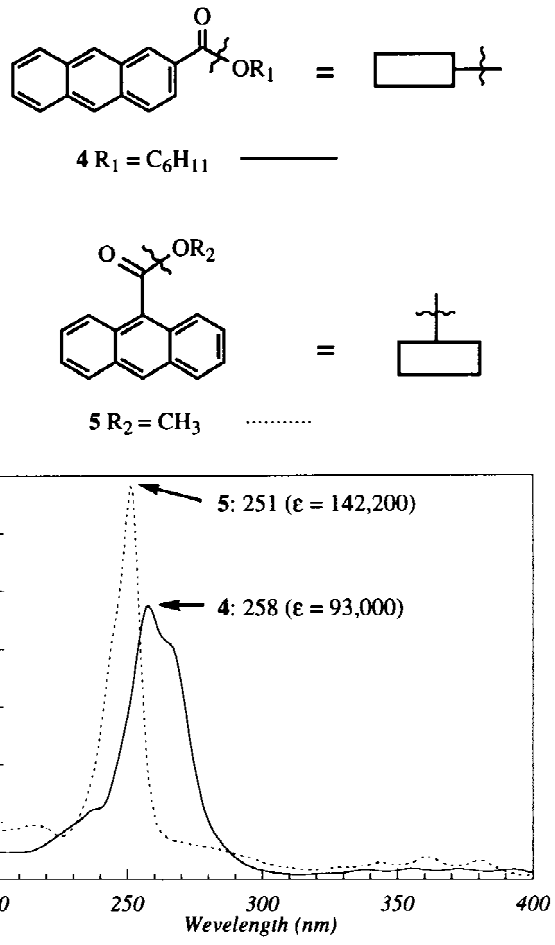
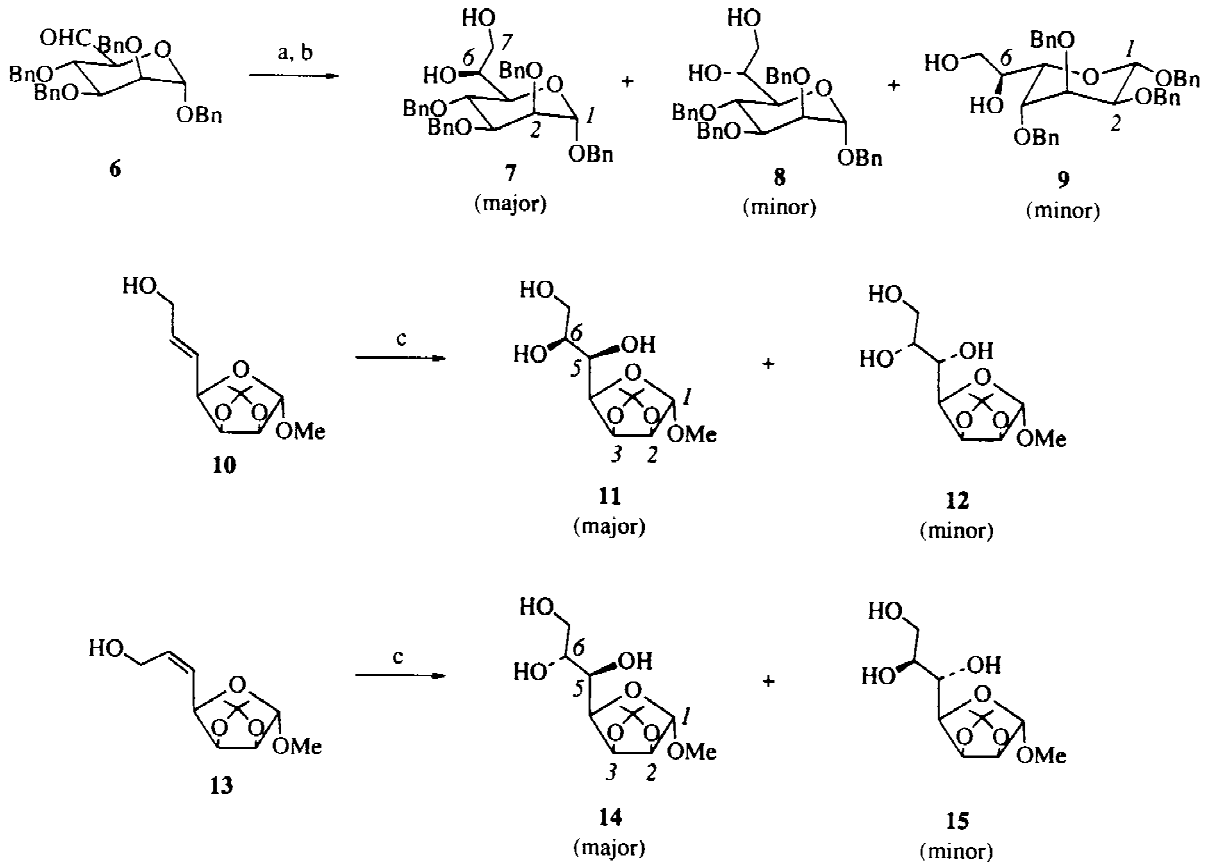


Fig. 2. UV spectra, in MeCN, of cyclohexyl-2-anthroate **4** (solid line) and methyl-9-anthroate **5** (dotted line).



Scheme 1. a: allylOCH$_2$MgCl, THF, −30° to 25°C. **b:** (PPh$_3$)$_3$RhCl, Dabco. **c:** 4-methylmorpholine N-oxide (NMO), OsO$_4$, t-BuOH/H$_2$O.

Heptofuranosides[15] with the 5,6-*threo* configuration, **11** and **12** (Scheme 1), were prepared, following known procedures,[2] by osmylation of *trans* allyl alcohol **10.** Based on Kishi's osmylation rule[16] the major product **11** is expected to have the (6S, 5R) L-*glycero*-D-*manno* configuration and the minor product **12** the (6R, 5S) D-*glycero*-L-*gulo* configuration.

Erythro heptofuranosides, **14** and **15,** were prepared, similarly, by osmylation of *cis* allyl alcohol **13.** Based again on Kishi's rule, the major product **14** and the minor product **15** should have, respectively, the (6R, 5R) D-*glycero*-D-*manno* and the (6S, 5S) L-*glycero*-L-*gulo* configurations.

Although the absolute configuration at the newly formed C-6 of heptopyranoside **7** is expected by Cram's rule to be L-*glycero,* further confirmation of its absolute configuration is necessary. Heptofuranosides **11** and **14** are expected by Kishi's rule to have the 5R/6S and 5R/6R configurations, respectively, as major products of the respective osmylation reactions but it is known that there are exceptions in Kishi's rule,[16,2] thus necessitating an independent proof of structure. Additionally, configuration at C-6 of compound **9** is completely unknown and requires assignment.

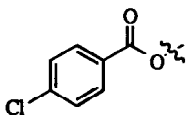
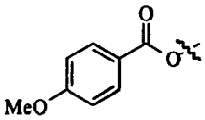
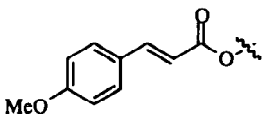
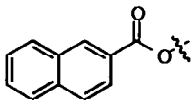
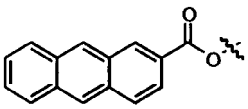
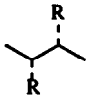
RESULTS AND DISCUSSION

As a part of a project to develop powerful CD chromophores with intense fluorescence and UV absorptions,[17] we have employed 2-naphthoate[18,19] and naphthimido[20] chromophores for the side chain derivatizations of two important classes of compounds, the brassinosteroids[19] and sphingosines.[20] Our continued search for better chromophores have led us to 2-anthroates prepared from the commercially available 2-anthroic acid; according to preliminary fluorescent studies, the sensitivity of 2-anthroate is twelvefold that of 2-naphthoate.

2-Anthroate, similar to 2-naphthoate, is a chromophore of lower symmetry than p-substituted benzoates. However, as in 2-naphthoate,[21] the orientation of the strong electric transition moment,[1]B$_b$, is almost parallel to the long axis of the chromophore and therefore almost parallel to the C-O bond.[22] This allows application of 2-anthroic acid for derivatization of alcohols in absolute configurational studies by the exciton chirality method.

The ε values and UV maxima of various monochromophoric derivatives are shown in Table 1. In order to compare the CD properties of these chromophores, the simple acyclic diol (2R,3R)-2,3-butanediol **16** was derivatized with various chromophores as shown in Table 1. Bis-2-anthroate derivative **17e** has the strongest UV absorption with ε = 147,000; bis-2-naphthoate derivative **17d** has ε = 108,000, while bis-p-chlorobenzoate derivative **17a** has only ε = 33,000. A similar trend is observed for the CD data. In all cases the sign of the CD spectra was the same but the CD amplitude of bis-2-anthroate derivative **17e,** −322, is far stronger than the others, e.g., bis-2-naphthoate **17d,** A =

TABLE 1. UV and CD data of various (2R,3R)-2,3-butanediol 16 derivatives

					
R	ε_{240} 21,400	ε_{257} 20,400	ε_{306} 23,400	ε_{232} 58,000	ε_{258} 93,000
	17a ε_{240} 33,900[a,b] 235 nm (+4) 250 nm (−6)	17b ε_{255} 36,100[a,c] 250 nm (+10) 268 nm (−7)	17c ε_{307} 45,000[d] 278 nm (+14) 317 nm (−22)	17d ε_{232} 108,000[e] 229 nm (+99) 242 nm (−116)	17e ε_{258} 147,000[e] 253 nm (+170) 274 nm (−152)
16 R = OH	**A = −10**	**A = −17**	**A = −36**	**A = −215**	**A = −322**

[a]UV and CD data were taken from Shapiro, 1996.[5]
[b]UV and CD spectra were measured in dioxane.
[c]UV and CD spectra were measured in EtOH.
[d]UV and CD spectra were measured in Me-C_6H_{11}.
[e]UV and CD spectra were measured in MeCN.

−215. It is also 10–30-fold stronger compared to the A values of other derivatives.

The combination of intense fluorescence together with strong UV absorptions and large CD amplitudes makes the 2-anthroate an excellent chromophore for microscale analysis and CD studies.

Exciton Coupling Between Identical Chromophores: 2-Anthroate/2-Anthroate Derivatives

Terminal 1,2-diols The terminal acyclic 1,2-diol is a common moiety in many natural products and synthetic intermediates, and assignment of the absolute configuration of the chiral center is very important. A simple approach for the determination of the absolute configuration of such systems employing the exciton chirality method is described by Harada et al.[7] Based on this method, the 1,2-diols are derivatized with p-substituted benzoate chromophores and the CD of the derivatives are measured. Derivatization, for example, of (2S)-1,2-propanediol **18** (Fig. 3) with p-bromobenzoate furnishes compound **19a,** which gives a positive CD bisignate curve in EtOH, 236 nm (−6)/253 nm (+15), A = +21.[7]

Compound **19a** can exist in three distinct conformations, A, B, and C, of which A and B lead to opposite CD exciton couplets. The exciton coupling of conformer C should be almost zero and therefore this conformation can only influence the amplitude but not the sign of the bisignate curve. Of the remaining two conformers, A and B, conformer B is not expected to contribute much because of extra steric interactions between the methyl group and the chromophores. Proof that conformer A is the major contributor to the overall conformation of **19a** comes from the vicinal coupling constants of $J_{1,2}$ = 6.8 (*trans*) and 3.6 Hz (*gauche*), indicating that the dihedral angles between the two protons are different.[7] Harada et al. have also shown by MO theoretical calculations (MOPAC Ver. 7.0, AM1 program) that conformer A is the most stable.[7]

Bis-2-anthroylated compound **19b** also exhibited a positive, but very intense, bisignate curve, which can be explained by conformer A being the predominant conformer ($J_{1,2}$ = 6.8 and 3.7 Hz). Compound **19b** has a strong UV absorption, in MeCN, at 258 nm with ε = 147,000 (Fig. 3). The ε value derived from the average of three exact weight measurements, is four times that of **19a** (λ_{max} 244, ε = 37,200 in EtOH). This intense absorption is clearly reflected in the CD amplitudes. Namely, the A value of compound **19b,** +190 (in MeCN), is almost ten times that of **19a** +21 (in EtOH).

The strong fluorescence, UV and CD spectra of 2-anthroate derivatives demonstrated this chromophore to be an excellent choice for microscale manipulations. In a model study, 40 ng of (2S)-1,2-propanediol **18** was anthroylated with 2-anthroyl-imidazole, purified by HPLC using UV detection[23] and provided sufficient material for UV and CD studies.

Thus, the sign of the bisignate curve for derivatized 1,2 diols with one stereogenic center remains consistent regardless of the substituents, indicating a strong preference for one conformation, namely conformation A. However, depending on substituents, conformers B and C could also contribute and give rise to lower amplitudes in CD. Nevertheless, in all known examples,[7,8] including the present results with 2-anthroate, conformation A is always the predominant one. It follows that the absolute configurations of terminal 1,2-diol systems can be unambiguously determined solely on the basis of the CD of 2-anthroates and other similar derivatives without NMR measurements.

Internal 1,2-diols Derivatization of vicinal diols with the same chromophore has also been used for assignment of absolute configurations of diols with two stereogenic centers. However, the CD spectra of these compounds show inconsistencies because the conformation and therefore the sign of the bisignate curve are greatly affected by the substituents in neighboring atoms. It was shown, for example, by Harada et al.[7] that the presence of bulky or polar substituents of the p-substituted benzoate derivatives of 1,2 diols with *threo* relative configuration might give CD signs opposite to those with non-polar or small substituents. This is attributed to different contributions of the conformers A, B, and C (illustrated in Fig. 4 for bis-2-anthroyl derivatives of **16, 20a,** and **20b**) and in such

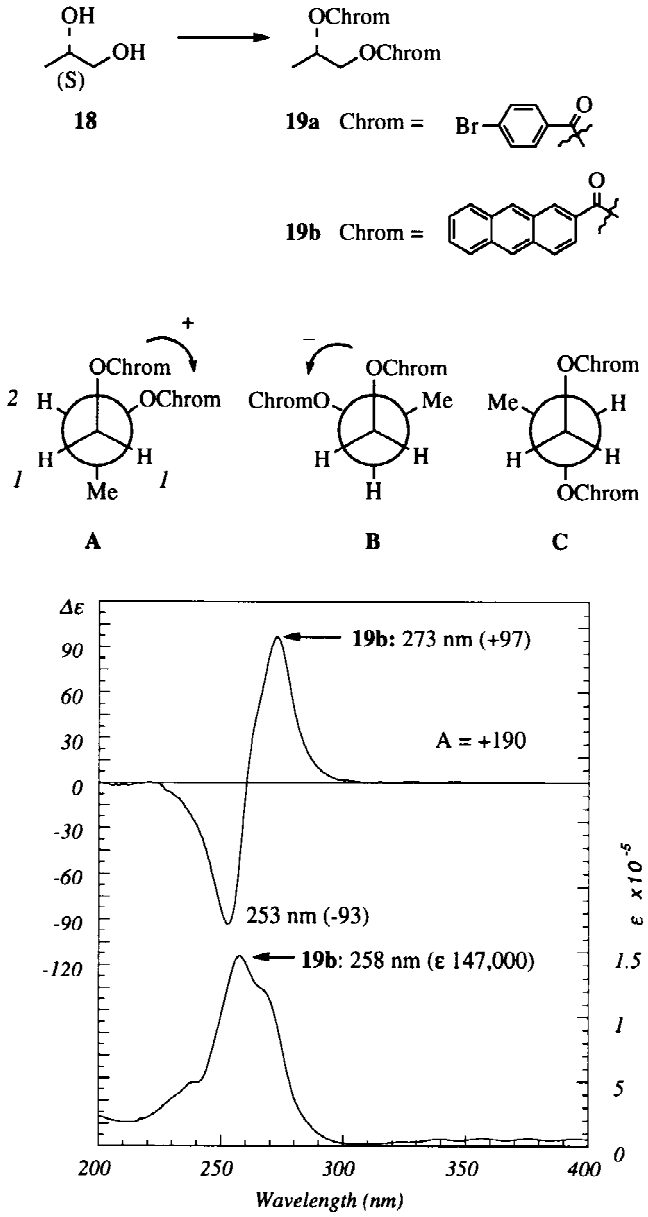


Fig. 3. Conformations of derivatives **19a** and **19b** and UV and CD spectra, in MeCN, of bis-2-anthroyl-(2S)-1,2-propanediol **19b.**

instances the preferred conformation can be determined from the vicinal proton-proton NMR coupling constant. For conformer A, the coupling constant is expected to be large (J_{vic} *trans* in the range of 6–8 Hz) while for conformer B it should be small (J_{vic} *gauche* around 3 Hz). When the substituents are small and non-polar, conformer A predominates due to the minimum number of steric interactions; however, bulky or polar substituents could result in destabilization of conformer A, leading to conformer B in which R_1 and R_2 are distal. Conformer C is not expected to contribute much, in either case, because of the increased number of steric interactions and proximity of the R_1 and R_2 groups.

We have recently employed these combined CD and NMR studies for the determination of the absolute configuration of brassinosteroids using 2-naphthoate as the chromophore.[19] The acyclic side chain of bioactive synthetic brassinosteroids contains two vicinal hydroxyl groups that could be either 22*R*/23*R* or 22*S*/23*S* (the 22*R*/23*S* or 22*S*/23*R* series have negligible activity). In a certain compound of this series with $J_{22,23}$ 8.8 Hz, conformer A showed to be the predominant, since its CD spectrum exhibited a strong negative bisignate curve, A = −424, this compound was assigned the 22*R*/23*R threo* configuration.

In the case of 2-anthroates, we have observed a similar

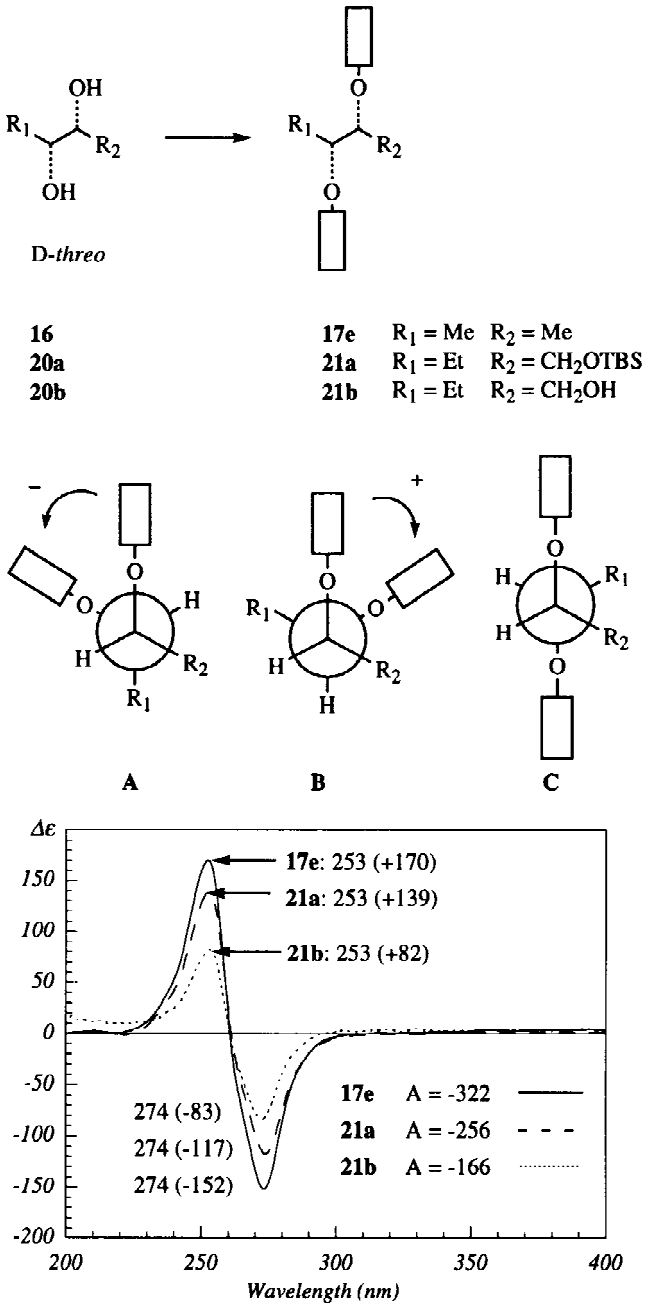


Fig. 4. CD spectra, in MeCN, of bis-2-anthroyl-(2R,3R)-2,3-butanediol **17e** (solid line), bis-2-anthroyl-(2R,3R)-1-*t*-BuMe_2Si-2,3-pentanetriol **21a** (dashed line), and bis-2-anthroyl-(2R,3R)-2,3-pentanetriol **21b** (dotted line).

pattern to other chromophores, except that the differences are clearer due to the more intense CD amplitudes. Model compounds of the D-*threo* configuration **16, 20a,** and **20b** gave derivatives **17e, 21a,** and **21b,** all of which exhibited strong negative couplets, A = −322, A = −256, A = −166, respectively. The advantages of 2-anthroyl derivatives over other chromophores are shown in Table 1. In all these examples, the sign of the bisignate curve is negative because the substituents are small and conformer A predominates. However, the difference in amplitudes of derivatives **17e, 21a,** and **21b** reflects various degrees of contribution from the other conformers B and C (and other factors such as difference in dihedral angle). The effect of substituents is seen clearly in compounds **17e** and **21b** where the amplitude of the CD curve of the former is double that of the later.

In the *erythro* series, conformer C (Fig. 5) with the minimum number of steric interactions is expected to be predominant, while conformers A and B should contribute less and equally to the overall conformation of the molecule. Based on these predictions, compounds of the *erythro* configuration are expected to have weak exciton coupling due to the almost *anti*-periplanar arrangement of the chromophores in conformer C. Indeed, bis-2-anthroylation of **22a** gave compound **23a,** which exhibits a negligible CD (Fig. 5). The CD of a similar compound differing only in the R_1 of **23a** ($R_1 = -(CH_2)_4OBn$) has been measured by Harada et al.[24] to also have a very weak CD signal. Compound **23b,** on the other hand, gives a strong positive bisignate curve (A = +189), indicating that, in this case, conformer B predominates possibly due to hydrogen bonding between the free primary hydroxyl group hydrogen and the C-3 ester oxygen; this hydrogen bonding is possible in conformers B and C but not in conformer A, therefore leading to the positive bisignate curve.

The above-mentioned monochromophoric CD/NMR method for determining the absolute configuration of acyclic 1,2 diols with the *threo* configuration has the advantage of simplicity in manipulations. However, in the case of *erythro* compounds (Fig. 5), prediction of conformation from NMR coupling constants alone is not possible since conformers A and B both give rise to small vicinal coupling constants; additional NOE measurements are required. In summary, in the general case with internal *vic*-diols, the CD data need to be supported by NMR coupling constants *and* NOE measurements.

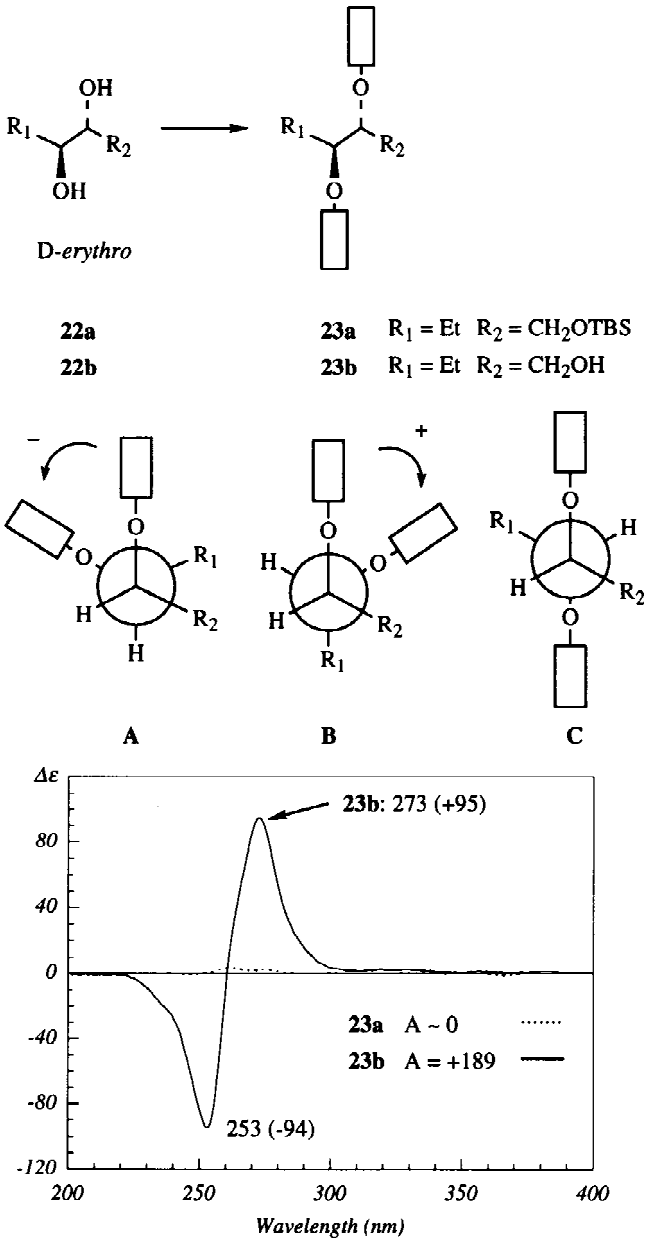


Fig. 5. CD spectra, in MeCN, of bis-2-anthroyl-(2S,3R)-2,3-pentanetriol **23a** (solid line) and bis-2-anthroyl-(2S,3R)-1-*t*-$BuMe_2Si$-2,3-pentanetriol **23b** (dotted line).

Exciton Coupling Between Different Chromophores: 9-Anthroate/2-Anthroate Derivatives

The bichromophoric approach has been proved so far to be the most successful for the assignment of the absolute configuration of acyclic polyols of both types: 1,2 and 1,2/1,3 mixed polyols. Previously,[9–11] acyclic polyols with a 1,2-diol terminal were derivatized selectively at the primary hydroxy group with 9-anthroate and at all other hydroxyl groups with p-methoxycinnamate, leading to "fingerprint" characteristic bisignate curves with A values in the range of 10–50. The energy difference between the absorption maxima and the difference in molecular absorptivities (λ_{max} = 258 nm, ε = 142,200 for 9-anthroate; λ_{max} = 306 nm, ε = 23,400 for p-methoxycinnamate) of the two chromophores were responsible for the characteristic "fingerprint" CD curves. Each reference compound gave a distinct CD pattern based on the different constituent interactions between the chromophores. However, one drawback of the 9-anthroate/p-methoxycinnamate combination is the much lower intensity of the first component of the exciton couplet at the longer wavelength, due to the relatively small ε value for the p-methoxycinnamate chromophore.

When the 9-anthroate/p-methoxycinnamate combination is replaced by the 9-anthroate/2-anthroate pair, the absorption maxima of the two chromophores are closer (λ_{max} = 253 nm, ε = 142,200 for 9-anthroate, λ_{max} = 258 nm,

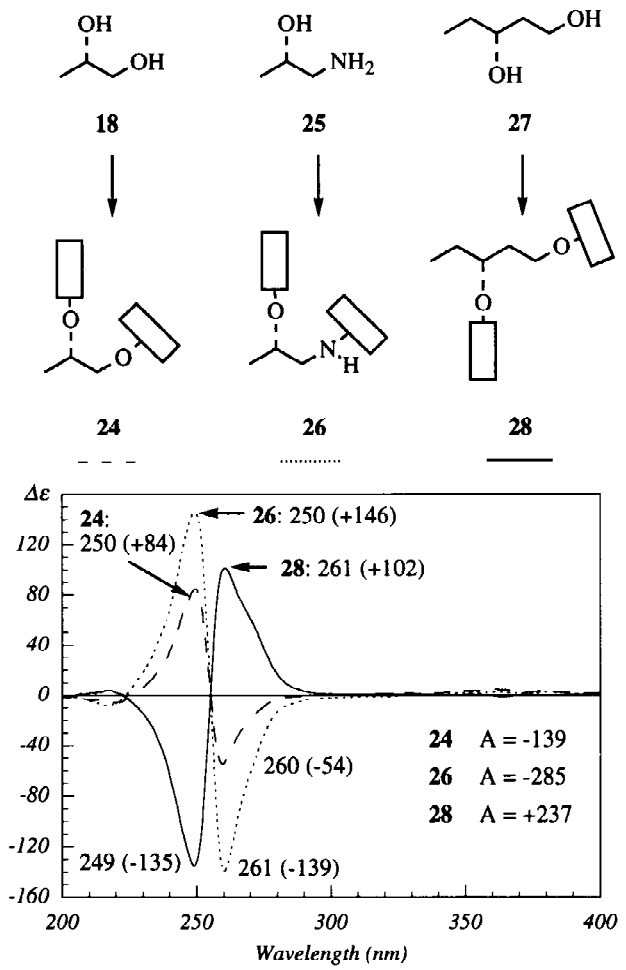


Fig. 6. CD Spectra, in MeCN, of 9-anthroyl-2-anthroyl-(2S)-propane-1,2-diol **24** (dashed line), 9-anthroyl-2-anthroyl-(2S)-1-amino-2-propanol **26** (dotted line), and 9-anthroyl-2-anthroyl-(2R)-butane-1,3-diol **28** (solid line).

ε = 93,000 for 2-anthroates, see Fig. 2), resulting in stronger coupling and more symmetrical shape of the bisignate curve.

Additionally, introduction of 2-anthroate as another highly fluorescent chromophore ensures high fluorescence in any medium because it was shown that, for example, methyl 9-anthroate has higher quantum yields in non-polar solvents and lower in polar solvents, while the opposite is true for the methyl 2-anthroate.[25]

1,2- and 1,3-Diols and 1,2-amino alcohol. We have first selected simple chiral acyclic diols, **18** and **27** (Fig. 6), and amino alcohol **25** to indicate the advantages of this new combination. Thus, 9-anthroylation, selectively at the primary hydroxyl groups of (*S*)-2-hydroxy-propanol **18,** (*S*)-2-hydroxy-propylamine **25,** and (R)-3-hydroxy-butanol **27,** followed by 2-anthroylation at the secondary hydroxyl groups, gave the corresponding derivatives **24, 26,** and **28** whose CD spectra are shown in Figure 6. The ε value of the bichromophoric derivative **24** was determined by averaging three exact weight measurements to be 184,000. The general trend in all spectra is the same as before[9–11] but the magnitude and the shape of the bisignate curves are different; namely, the new chromophore pair gives large A values and clearly more symmetric bisignate curves. Compound **24** gave a negative bisignate curve at 250 nm (+84)/260 nm (−54), A = −139, which is much larger than that of 9-anthroate/p-methoxycinnamate derivative of **18** 252 nm (+28)/295 nm (−5), A = −33.[11] A similar trend is observed with compounds **26** and **28.** Compound **28,** for example, gave a positive bisignate curve (A = +237), which is ten times larger than previous examples with p-methoxycinnamate (unpublished results). The difference in amplitudes between derivatives **24** (A = −139) and **26** (A = −285) can be explained by a more rigid conformation in **26** due to the amide bond.[11]

It should be noted that although the CD spectra of mono- and bi-chromophoric derivatives of (2*S*)-1,2-propanediol **18,** namely, bis-2-anthroate **19b** (Fig. 3) and 9-anthroate-2-anthroate **24** (Fig. 6) exhibit a positive and negative couplet, respectively, the two results are not in contradiction. The difference in signs are due to the different relative orientation of the strong ^1B_b electric transition moments in the 9-anthroate and the 2-anthroate chromophores (Fig. 7).

Triols. Derivatization of model acyclic D-*erythro*-triol **22** (Fig. 8) and D-*threo*-triol **20,** with the 9-anthroate/2-anthroate combination, gave compounds **29** and **30,** respectively. The ε value of compound **30,** i.e., 235,000, was the average of three exact weight measurements. The derivatives exhibited strong characteristic CD curves that are distinctly different from each other.

The CD spectrum of the *erythro* compound **29** consists mostly of the strong 1,2 and 1,3-interactions between the 9-anthroate (λ_{max} 251 nm) and the 2-anthroates (λ_{max} 258 nm) as the extrema at 259 nm (−101)/249 nm (+113) indicate, and only a weak interaction between the two 2-anthroyl components (small positive Cotton effect at 276 nm, $\Delta\varepsilon$ = +23). A similar trend has been observed before with the 9-anthroate/p-methoxycinnamate derivative of **22**[11c] where an asymmetric couplet was observed at 278 nm (−14)/252 nm (+27), originating from p-methoxycinnamate (λ_{max} 306 nm) and 9-anthroate (λ_{max} 251 nm) coupling, with the 9-anthroate component of the couplet being much stronger due to the large difference in ε values be-

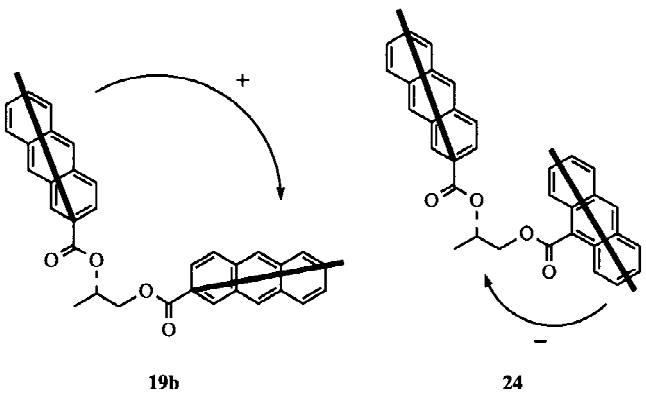


Fig. 7. The orientation of the strong ^1B_b electric transition moments in bis-2-anthroyl-(2S)-propane-1,2-diol **19b** and 9-anthroyl-2-anthroyl-(2S)-propane-1,2-diol **24.**

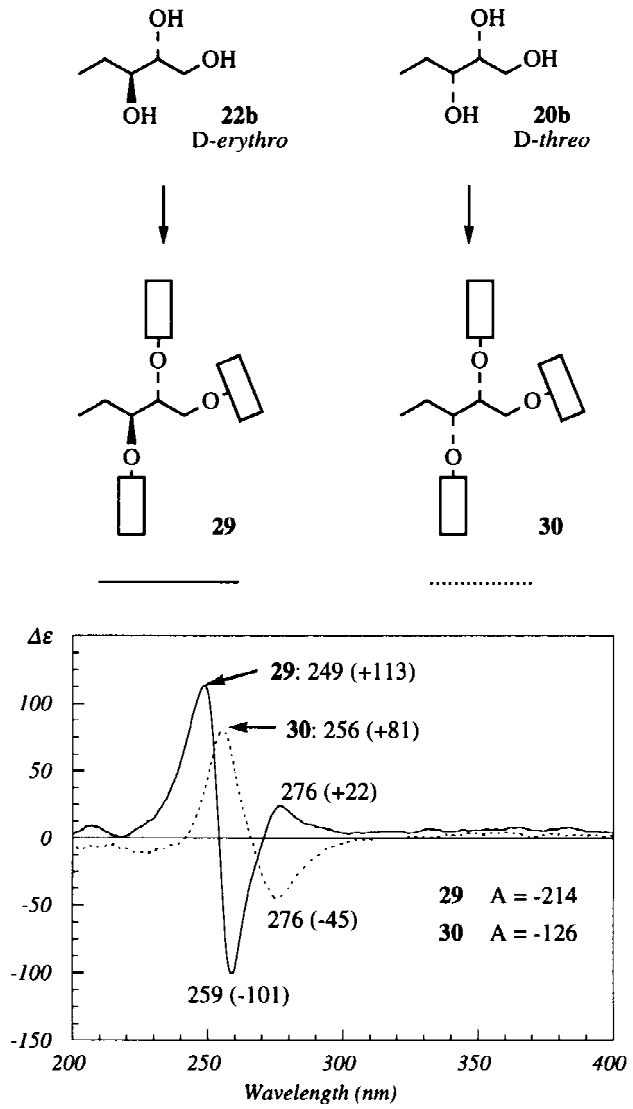


Fig. 8. CD spectra, in MeCN, of 9-anthroyl-2-anthroyl- (2R,3S)-1,2,3-pentanetriol **29** (solid line) and 9-anthroyl-2-anthroyl-(2R,3R)-1,2,3-pentanetriol **30** (dotted line).

tween 9-anthroate and p-methoxycinnamate; the observed weak positive Cotton effect at 319 nm (+4) originated from p-methoxycinnamate interactions.[11c]

In the *threo* compound **30,** the interactions between 9-anthroate and 2-anthroates almost cancel each other and the major contribution in the CD spectrum originates from the 2,3 coupling between the two 2-anthroates, 275 nm, (−45)/256 nm (+81), A = −126. For comparison, the 9-anthroate/p-methoxycinnamate derivative of **20b** also exhibited a negative bisignate curve, 318 nm (−4)/280 nm (+8), A = −12.[11c]

Thus, 9-anthroate/2-anthroate combination retains all the information necessary to assign the structure of a 1,2,3 triols and provides, at the same time, much higher amplitudes and clear-cut symmetrical bisignate curves in contrast to the previous 9-anthroate/p-methoxycinnamate combination.[11c] However, *threo* 1,2,3 triols carrying bulky substituents such as **34** may exhibit significantly different CD spectra.

Determination of the Absolute Configuration of the Side Chain of Heptopyranosides and Heptofuranosides

The bichromophoric method has been successfully applied to a variety of compounds, including bacteriohopanoids.[26] However, all the compounds used so far were fairly simple and assignments of the absolute stereochemistry in more complex acyclic polyols still remain one of the more difficult and challenging tasks in structural organic chemistry.[4a] Heptoses such **1, 2,** and **3** (Fig. 1) having bulky substituents in close proximity to the acyclic chain represent such a challenge for structural elucidations. Ongoing investigation on their structure and properties led to the synthesis of several heptopyranosides **7–9** and heptofuranosides **11, 12, 14,** and **15** (Scheme 1). Because of their bulky side chains, these sugars provide suitable substrates for testing the exciton chirality method on more complex polyols.

We started our studies with compound **7** prepared upon addition of allyloxymethyl magnesium chloride to D-*manno*-aldehyde **6.** Since compound **7** was isolated as the Cram major product of the reaction, it was expected to be a (6S) or L-*glycero* D-*manno*-pyrannoside (as depicted). Based on the bichromophoric 9-anthroate/2-anthroate method, we expected a positive bisignate curve, opposite to model compound **24** (Fig. 6). Indeed, derivative **31** (Fig. 9) exhibited a strong positive CD couplet (A = +188), suggesting the (6*S*) configuration. Additionally, bisderivatization of **7** with 2-anthroate gave compound **32,** which exhibits an expected negative bisignate curve (A = −285), again suggesting the (6*S*) configuration. This is in accordance with the spectrum of model compound **19b** (Fig. 3) with 2*S* configuration exhibiting a positive couplet. Thus, the CD data unambiguously confirmed that compound **7** has the (6*S*) or L-*glycero* D-*manno*-configuration.

The determination of the stereochemistry at position 6 of the "inverted" pyranoside **9** (Scheme 1) was more challenging. The bichromophoric approach proved to be unsuited for this compound, neither after 9-anthroate/2-anthroate nor after 9-anthroate/p-methoxycinnamate derivatizations. In both cases, we observed uncharacteristic Cotton effects (nm/($\Delta\varepsilon$)) as the following: (262 nm (−13), 247 nm (−3.8) for the 9-anthroate/2-anthroate derivative and 251 nm (−3.4), 232 nm (+4.7) for 9-anthroate/p-methoxycinnamate of **9.** These CD curves bear no resemblance to any of the corresponding known reference curves, presumably due to an unknown and unique conformation of the molecule.

We then turned to derivatization with identical chromophores. The 2-anthroate/2-anthroate method indeed proved better and compound **33** (Fig. 9) provided a relatively weak negative bisignate curve (A = −50). The sign of the bisignate curve is the same as that of compound **32** (A = −285), suggesting that the configuration at C-6 of the "inverted" derivative **33** is the same as that of C-6 in derivative **32,** i.e., (6*S*) or L-*glycero*. Thus, the L-*glycero*-L-*gulo* configuration was assigned to compound **9** in which the C-6 configuration was previously unknown.

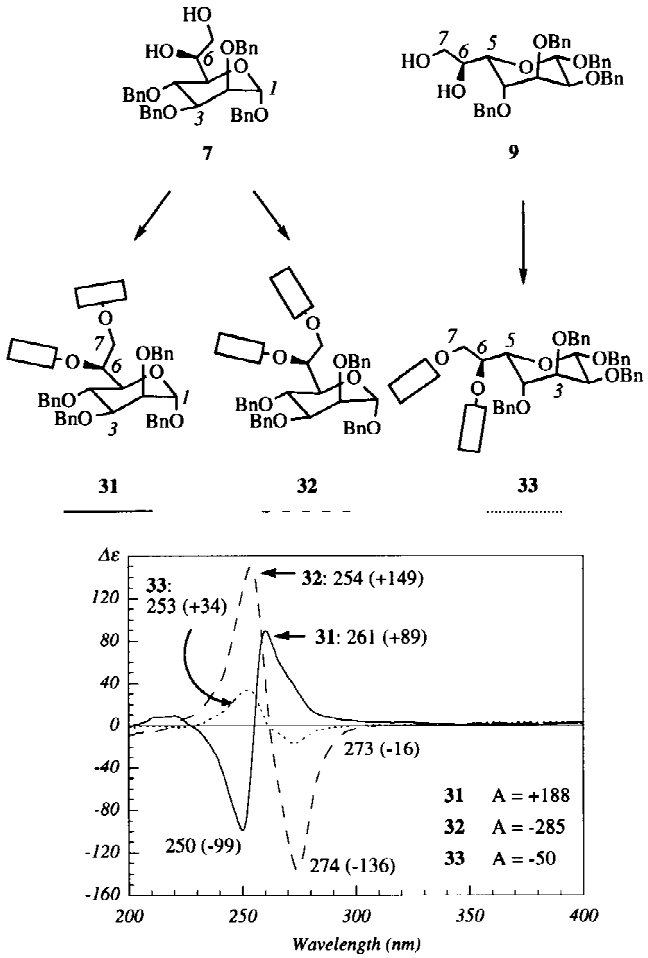


Fig. 9. CD spectra, in MeCN, of **31** (solid line), **32** (dashed line), and **33** (dotted line).

The weak negative amplitude of the CD of compound **33** (side chain configuration is opposite to that of **18,** Fig. 3) suggests an increase in the population of conformer B (Fig. 3, *mirror image!*) thus resulting in a *positive* contribution to the negative exciton coupling. NMR coupling constants indicated a significant decrease in the *trans* 6-H/7-H coupling $J_{6,7}$ = 4.9 Hz, which usually is in the range of 8 Hz, while the *gauche* 6-H/7-H remained normal ($J_{6,7}$ = 2.2 Hz).

Harada et al.[7] have observed similar effects where the conformation equilibrium depends on substituents; for example, the change in compound **19a** (Fig. 3) from a methyl to a benzyl substituent results in a change in J and CD amplitudes from $J_{1,2}$ = 6.8 Hz, A = +20.3 to $J_{1,2}$ = 8.4 Hz, A = +28.5.

The influence of substituents on the conformation of the molecule was more dramatically seen in the case of heptofuranoside **11.** Based on the synthetic scheme, this compound is expected to have the (5*R*, 6*S*) or L-*glycero*-α-D-*manno* configuration. However, this expectation was not supported by the bichromophoric method when compound **11** was derivatized with the 9-anthroate/bis-2-anthroate combination to provide derivative **34** (Fig. 10). Compound **34** gave a negative exciton couplet, 252 nm (+70)/274 nm (−93), A = −163 (CD not shown), which was similar to that of model triol **30** (Fig. 8), suggesting a (5*S*,6*R*) or D-*glycero*-β-L-*gulo* configuration. This is contradictory to the prediction based on synthesis.

A rational of these results stems from the fact that the CD spectrum of **34** derives mainly from the interaction between the two 2-anthroates. As discussed earlier, it is important to determine the major conformation in internal *threo* 1,2-diols before using CD for configurational assignments. Since the exciton couplet of **34** is negative, there are two *threo* conformations that can lead to such a sign; one is conformation A (Fig. 4), leading to 5*S*/6*R* stereochemistry, while the other is the mirror image of conformation B, in which case the stereochemistry is 5*R*/6*S*. The $J_{5,6}$ of **34** is 2.3 Hz, indicating a *gauche* orientation of the two hydrogens, i.e., the preferred conformation of this molecule in solution is the mirror image of conformation B.

Similarly, the previously developed 9-anthroate/bis-p-

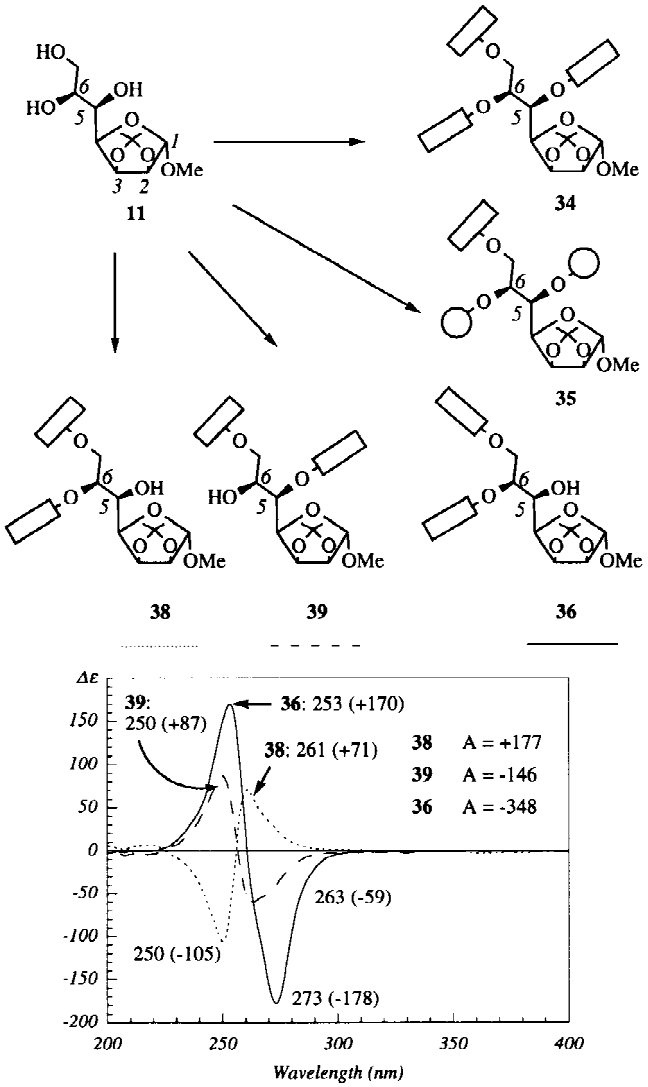
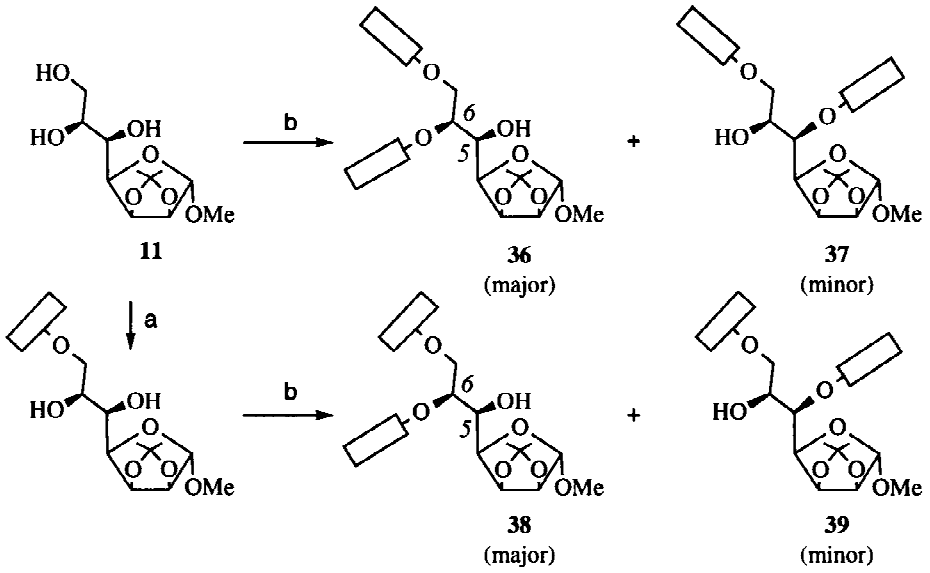


Fig. 10. Derivatives **34, 35, 38, 39,** and **36.** Circles in **35** represent the p-methoxycinnamate chromophore. CD spectra, in MeCN, of compounds **38** (dotted line), **39** (dashed line), and **36** (solid line).



Scheme 2. a: 9-Anthroylfluoride, pyridine, DMAP. **b:** 2-Anthroylimidazole, DBU, MeCN.

methoxycinnamate method also gave unclear results providing compound **35** with a weak CD (not shown), 252 nm (−6)/272 nm (+1)/308 nm (−2) that was difficult to interpret. Compound **11** is the first example of a complex triol in which assignment of the absolute configuration based solely on the bichromophoric method failed; this shows that this protocol should be used with caution when the substituents are not simple acyclic chains.

In order to overcome the above problems, a different route was taken to determine the side chain configuration of compound **11,** which, according to the synthesis, should be a *threo* compound. Derivatization of the primary hydroxyl group of **11** with 9-anthroate and monoderivatization with 2-anthroate led to compounds **38** and **39** (Scheme 2). The position of the 2-anthroate chromophore (5-OH or 6-OH) was determined by NMR, based on the downfield chemical shift of proton 5-H or 6-H attached to the acylated carbon. Compound **38,** a terminal 1,2 diol ester, gave a positive bisignate curve (A = +177) (Fig. 10) similar to that of compound **31** (A = +188) (Fig. 9) and opposite to the reference compound **24** (Fig. 6), suggesting that the stereochemistry at C-6 is the same as in compound **31,** i.e., S or L-*glycero.* Since we know that the relative configuration at C-5 and C-6 is *threo,* it follows that the stereochemistry at C-5 should be R. Additional proof for the stereochemistry of C-5 comes from compound **39,** a terminal 1,3 diol; this compound gave a negative CD curve (A = −146), which is opposite to that of model compound **28** (A = +237) (Fig. 6); therefore, the stereochemistry at C-5 is *R.*

Similarly, partial derivatization of **11** with 2-anthroate gave compounds **36** and **37** (Scheme 2). Compound **36** gave a strong negative CD (A = −348) similar to that of compound **32** (A = −285) (Fig. 9). Therefore, the two compounds have the same stereochemistry at C-6, i.e., (6*S*) or L-*glycero.* Compound **37** (CD not shown) gave a very weak CD that was not interpretable. In conclusion, the absolute configuration of **11,** originally proposed by synthetic/stereochemical considerations, was confirmed by the exciton chirality method to be (5*R,* 6*S*) or L-*glycero*-D-*manno.*

The CD analysis of *threo* 1,2,3-triols derivatives containing two types of chromophores has shown that it is the pair-wise interaction between the two internal 2-anthroate chromophores that determines the overall shape of the CD curves, and that the terminal 9-anthroate has no effect. Moreover, this interaction strongly depends on the conformational distribution of the vicinal bis 2-anthroate moiety, which in turn is influenced by the steric size of the substituent at C-3. For this reason, the CD spectra of threo 1,2,3-triols bichromophoric derivatives such as **30** (Fig. 8) and **34** (Fig. 10 and the following data) are less characteristic and unreliable as references for absolute stereochemistry. For example, the CD of triol derivative **34,** linked to the bulky furanoside ring, exhibits a negative couplet, 274 nm (−93)/252 nm (+70) (in acetonitrile); this resembles the negative couplet of derivative **30,** 276 nm (−45)/256 nm (+81) (Fig. 8), the absolute configuration of which is opposite. In contrast, the CD spectra of *erythro* 1,2,3-triols are more characteristic and stable for a given configurational type; i.e., their overall CD curves are distinctive for all chromophoric interactions involved, including interactions with the 9-anthroate group at C-1.

Thus, derivatization of *erythro* compound **14** (Scheme 1) by 9-anthroate/bis-2-anthroate provided compound **40** (Fig. 11), which exhibited a negative bisignate CD, 249 nm (+177)/259 nm (−157) A = −334, very similar to that of model compound **29** (Fig. 8) and, therefore, compound **14** was assigned the (5R, 6R) or D-*glycero*-D-*manno* configuration. This conclusion was corroborated by the CD of 9-anthroate/bis-p-methoxycinnamate derivative **41,** which exhibited a negative bisignate curve, 253 nm (+39)/286 nm (−13), A = −52, similar to that of the 9-anthroate/bis-p-

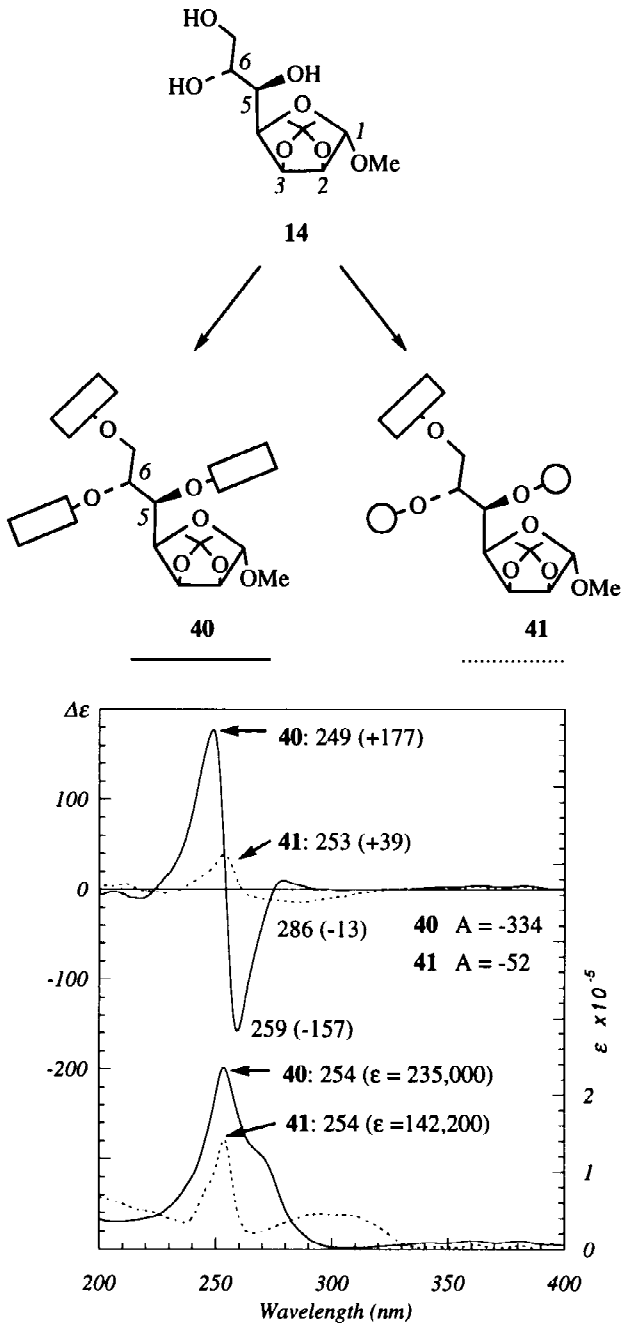


Fig. 11. UV and CD spectra, in MeCN, of **40** (solid line) and **41** (dotted line). Circles in **41** represent the p-methoxycinnamate chromophore.

methoxycinnamate derivative of **22.**[11c] Figure 11 exemplifies the differences between the 9-anthroate/2-anthroate and 9-anthroate/p-methoxycinnamate methods. Both the UV and CD spectra of **40** are much stronger than that of **41.** In addition, the shape of the bisignate curve of **40** is more symmetric, with clear positive and negative Cotton effects, while in **41** the negative Cotton effect is weak. The amplitude of the CD spectrum of **40** (A = −334) is sixfold that of **41** (A = −52). These differences are particularly significant when only a limited amount of starting material is available.

CONCLUSION

In conclusion, a new chromophore for the determination of the relative and absolute configuration of diols, aminols, and triols has been introduced; it allows microscale detection by fluorescence, UV, and CD. The excellent properties of the 2-anthroate chromophore greatly facilitate the interpretation of spectra, especially those of acyclic systems that lack rigidity and, hence, should be interpreted with caution. We are currently investigating further applications of this chromophore.

EXPERIMENTAL SECTION

General

All compounds were purchased from Aldrich (Milwaukee, WI) except 2-anthracene carboxylic acid, which was purchased from TCI America (Portland, OR). Methylene chloride was distilled from calcium hydride under nitrogen. Acetonitrile was Aldrich anhydrous grade for reactions and spectrophotometric grade for obtaining CD spectra. DMF was Aldrich anhydrous grade. Analytical and preparative TLC was run on precoated silica-gel plates (Analtech (Newarte, DE) 20 × 20 cm, 250 and 500 μm, respectively). Analytical and preparative HPLC was performed on Waters (Rochester, MN) or Rainin (Woburn, MA) HPLC. ^{1}H NMR spectra were recorded on a Bruker 500-MHz spectrometer. All chromophoric derivatives were submitted to HPLC purification and MS analysis prior to UV/CD measurements. UV spectra were taken on a Perkin-Elmer (Oak Brook, IL) Lambda 6 model. CD analysis was performed on a Jasco (Easton, MD) J-720 spectropolarimeter.

Preparation of anthroylimidazole. Carbonyldiimidazole (42 mg, 0.26 mmol) was added to a solution of anthroic acid (56 mg, 0.25 mmol) in anhydrous MeCN (2 mL) at room temperature and the mixture was stirred overnight to afford a yellow slurry. Upon evaporation of the solvent, the crude mixture was loaded to a short silica gel column and washed with CH_2Cl_2. The pure product was then eluted with ethyl acetate. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.58 (s, 1 H), 8.51 (s, 1 H), 8.50 (s, 1 H), 8.19 (s, 1 H), 8.15 (d, J = 8.8 Hz, 1 H), 8.05 (d, J = 9.3 Hz, 2 H), 7.79 (dd, J = 1.6 Hz, J = 8.8 Hz, 1 H), 7.64–7.63 (m, 1 H), 7.59–7.53 (m, 2 H), 7.21 (s, 1 H); MS/CI (M^+) 272.

Microscale anthroylation procedure. Anthroylimidazole (750 μg in 250 μL MeCN) and DBU (0.2 μL, in 5 μL MeCN) were added to 1 μg of diol and the resulting mixture was stirred for 3 h at room temperature. The solvent was then evaporated and the crude mixture was purified by plate chromatography and HPLC, using 10% ethyl acetate/hexanes as the solvent system, to yield 6.4 μg of product (96% yield).

(2S)-Bis-1,2-o-(2-anthroyl)-1,2-propanediol (19b). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.83 (s, 1 H), 8.79 (s, 1 H), 8.53 (s, 1 H), 8.46 (s, 1 H), 8.42 (s, 1 H), 8.40 (s, 1 H), 8.02–7.92 (m, 8 H), 7.52–7.45 (m, 4 H), 5.70–5.62 (m, 1 H,

H-2), 4.67 (dd, J = 3.7 Hz, J = 11.8 Hz, 1 H, H-1), 4.61 (dd, J = 6.8 Hz, J = 11.8 Hz, 1 H, H-1), 1.58 (d, J = 6.5 Hz, 3 H, H-3); MS/CI (M^+) 484.

o-(2-Anthroyl)-cyclohexanol (4) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.79 (s, 1 H), 8.56 (s, 1 H), 8.43 (s, 1 H), 8.03–7.99 (m, 4 H), 7.52–7.44 (m, 2 H), 5.13–5.08 (m, 1 H), 2.08–1.99 (m, 2 H), 1.85–1.82 (m, 2 H), 1.75–1.36 (m, 6 H); MS/CI (M^+) 304.

(2R, 3R)-Bis-2,3-o-(2-anthroyl)-2,3-butanediol (17e) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.79 (s, 2 H), 8.48 (s, 2 H), 8.36 (s, 2 H), 8.04–7.95 (m, 8 H), 7.52–7.45 (m, 4 H), 5.51–5.48 (m, 2 H), 1.52 (bs, 6 H); MS/CI (M^+) 498.

(2R,3S)-1-o-(*tert*-butyl-dimethylsilyl)-2,3-bis-o-(2-anthroyl)-1,2,3-pentanetriol (23a) A solution of (2R,3S)-1,2,3-pentanetriol (14 mg, 0.12 mmol) in DMF (1 mL) was treated with t-butyldimethylsilylchloride (20 mg, 0.13 mmol) and imidazole (18 mg, 0.26 mmol) and the resulting mixture was stirred overnight. It was then quenched with water, extracted with ethyl acetate, and dried under Na_2SO_4. Column chromatography (silica, 30% ethyl acetate/hexanes) provided the pure product, which was submitted to anthroylation with 2-anthroic acid (57 mg, 0.26 mmol), EDC (50 mg, 0.29 mmol), and DMAP (32 mg, 0.26 mmol) in CH_2Cl_2 (1 mL). After allowing the reaction mixture to stir overnight, plate chromatography (silica, 10% ethyl acetate/hexanes) provided the pure product (overall 50% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.79 (s, 1 H), 8.78 (s, 1 H), 8.48 (s, 1 H), 8.46 (s, 1 H), 8.44 (s, 2 H), 8.04–7.94 (m, 8 H), 7.53–7.47 (m, 4 H), 5.62–5.58 (m, 2 H, H-2, H-3), 4.11–4.03 (m, 2 H, H-1), 2.08–1.99 (m, 2 H, H-4), 1.09 (t, J = 7.35 Hz, 3 H, H-5), 0.86 (s, 9H, *t*-Bu-Si), 0.05 (s, 3 H, Me-Si), 0.02 (s, 3 H, Me-Si); MS/CI (M^+) 642. Deprotection of the primary hydroxyl group with TBAF in THF yielded quantitatively **(2R,3S)-2,3-bis-o-(2-anthroyl)-1,2,3-pentanetriol (23b):** MS/CI ($M^+ + H^+ + NH_3$) 546.

(2R,3R)-1-o-(*tert*-butyl-dimethylsilyl)-2,3-bis-o-(2-anthroyl)-1,2,3-pentanetriol (21a) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.80 (s, 1 H), 8.78 (s, 2 H), 8.48 (s, 1 H), 8.47 (s, 1 H), 8.35 (s, 1 H), 8.11–7.94 (m, 7 H), 7.50–7.44 (m, 5 H), 5.66–5.62 (m, 1 H), 5.53–5.50 (m, 1 H), 3.98–3.97 (m, 1 H, H-1), 2.05–1.90 (m, 2 H, H-4), 1.08 (t, J = 7.06 Hz, 3 H, H-5), 0.86 (s, 9H, *t*-Bu-Si), 0.02 (s, 3 H, Me-Si), 0.01 (s, 3 H, Me-Si); MS/CI ($M^+ + H^+ + NH_3$) 660, ($M^+ + H^+$) 643.

(2R,3S)-2,3-bis-o-(2-anthroyl)-1,2,3-pentanetriol (21b) 8.86 (s, 1 H), 8.73 (s, 1 H), 8.54 (s, 1 H), 8.43 (s, 1 H), 8.40 (s, 1 H), 8.38 (s, 1 H), 8.00–7.89 (m, 8 H), 7.55–7.46 (m, 4 H), 5.65–5.62 (m, 2 H, H-2, H-3), 4.89 (dd, J = 11.89 Hz, J = 4.6 Hz, 1 H, H-1), 4.72 (dd, J = 11.87 Hz, J = 7.25 Hz, 1 H, H-1), 2.00–1.95 (m, 2 H, H-4), 1.08 (t, 3 H, H-5); MS/CI ($M^+ + H^+ + NH_3$) 546.

(2S)-1-o-(9-anthroyl)-2-o-(2-anthroyl)-1,2-propanediol (24) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.85 (s, 1 H), 8.53 (s, 1 H), 8.49 (s, 1 H), 8.45 (s, 1H), 8.08–7.95 (m, 8 H), 7.53–7.26 (m, 6 H), 5.70–5.66 (m, 1 H), 4.91 (dd, J = 6.5 Hz, J = 11.9 Hz, 1 H), 4.85 (dd, J = 3.3 Hz, J = 11.9 Hz, 1 H), 1.57 (d, J = 6.5 Hz, 3 H); MS/CI (M^+) 484.

(2S)-1-o-(9-anthroyl)-2-o-(2-anthroyl)-1-amino-2-propanol (26) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.76 (s, 1 H), 8.48 (s, 1 H), 8.39 (s, 2 H), 7.98–7.90 (m, 8 H), 7.53–7.34 (m, 6 H), 6.45–6.42 (m, 1 H), 5.57–5.51 (m, 1 H), 4.12–4.00 (m, 2 H), 1.58 (d, J = 6.4 Hz, 3 H); MS/CI ($M^+ + NH_3$) 501.

(3R)-1-o-(9-anthroyl)-3-o-(2-anthroyl)-1,3-butanediol (28) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.64 (s, 1 H), 8.42 (s, 1 H), 8.39 (s, 2 H), 8.04–7.87 (m, 8 H), 7.54–7.40 (m, 6 H), 5.52–5.44 (m, 1 H), 4.86–4.81 (m, 1 H), 4.78–4.73 (m, 1 H), 2.45–2.35 (m, 1 H), 2.32–2.24 (m, 1 H), 1.49 (d, J = 6.3 Hz, 3 H); MS/CI ($M^+ + NH_3$) 516.

(2R,3S)-1-o-(9-anthroyl)-2,3-bis-o-(2-anthroyl)-1,2,3-pentanetriol (29) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.04 (s, 1 H), 8.82 (s, 1 H), 8.79 (s, 1 H), 8.76 (s, 1 H), 8.74 (s, 1 H), 8.69 (s, 1 H), 8.63 (s, 1 H), 8.16–7.86 (m, 10 H), 7.58–7.45 (m, 3 H), 7.31–7.28 (m, 4 H), 7.31–7.25 (m, 3 H), 5.99–5.92 (m, 1 H), 5.75–5.71 (m, 1 H), 5.18 (dd, J = 6.5 Hz, J = 12.1 Hz, 1 H), 5.09 (dd, J = 3.3 Hz, J = 12.1 Hz, 1 H), 2.07–2.01 (m, 2 H), 1.28 (t, J = 7.5 Hz, 3 H).

(2R,3R)-1-o-(9-anthroyl)-2,3-bis-o-(2-anthroyl)-1,2,3-pentanetriol (30) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.89 (s, 1 H), 8.62 (s, 1 H), 8.50 (s, 1 H), 8.42 (s, 1 H), 8.32 (s, 1 H), 8.29 (s, 1 H), 8.18 (s, 1 H), 8.05–7.95 (m, 10 H), 7.82–7.76 (m, 3 H), 7.51–7.50 (m, 4 H), 7.31–7.25 (m, 3 H), 5.95–5.93 (m, 1 H), 5.73–5.69 (m, 1 H), 5.17 (dd, J = 3.7 Hz, J = 12.3 Hz, 1 H), 4.95 (dd, J = 5.6 Hz, J = 12.3 Hz, 1 H), 2.03–1.96 (m, 2 H), 1.08 (t, J = 7.4 Hz, 3 H); MS/CI (M^+) 732.

Benzyl 6,7-bis-o-(2-anthroyl)-2,3,4-tris-o-benzyl-L-*glycero*-α-D-*manno*-pyranoside (32) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.90 (s, 1 H), 8.71 (s, 1 H), 8.41 (s, 1 H), 8.38 (s, 2 H), 8.36 (s, 1 H), 8.22–7.91 (m, 9 H), 7.56–7.13 (m, 23 H), 6.21–5.98 (m, 1 H), 5.14 (d, J = 1.5 Hz, 1 H), 4.89–4.62 (m, 9 H), 4.53 (dd, J = 9.9 Hz, J = 12 Hz, 1 H), 4.23–4.07 (m, 4 H), 3.89–3.86 (m, 1 H); MS/CI (M^+) 979.

Benzyl 6-o-(2-anthroyl)-7-o-(9-anthroyl)-2,3,4-tris-o-benzyl-L-*glycero*-α-D-*manno*-pyranoside (31) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.86 (s, 1 H), 8.44 (s, 1 H), 8.38 (s, 1 H), 8.03–7.91 (m, 10 H), 7.45–7.02 (m, 22 H), 6.21–5.98 (m, 1 H), 5.11 (bs, 1 H), 4.84–3.84 (m, 14 H); MS/CI (M-H^+) 978.

Benzyl 6,7-bis-o-(2-anthroyl)-2,3,4-tris-o-benzyl-L-*glycero*-α-L-*gulo*-pyranoside (33) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.73 (s, 1 H), 8.69 (s, 1 H), 8.46 (s, 1 H), 8.44 (s, 1 H), 8.40 (s, 1 H), 8.38 (s, 1 H), 8.02–7.89 (m, 10 H), 7.53–6.98 (m, 22 H), 5.82–5.79 (m, 1 H, H-6), 5.08 (d, J = 8.22 Hz, 1 H), 5.01 (dd, J = 12.49 Hz, J = 2.34 Hz, 1H, H-7), 4.98 (d, J = 12.37 Hz, 1 H), 4.89 (d, J = 12.09 Hz, 1H), 4.82 (d, J = 12.29 Hz, 1 H), 4.79 (dd, J = 12.30 Hz, J = 4.93 Hz, 1 H, H-7), 4.69 (d, J = 12.14 Hz, 1 H), 4.63 (d, J = 12.10 Hz, 1 H), 4.52 (d, J = 12.33 Hz, 1 H), 4.46 (d, J = 9.22 Hz, 1 H, H-5), 4.23 (q, J = 11.62 Hz, 2 H), 3.83 (dd, J = 3.33 Hz, 1 H, H-3), 3.73

(dd, J = 8.16 Hz, J = 3.08 Hz, 1 H, H-2), 3.54 (d, J = 2.77 Hz, 1 H, H-4); MS/CI (M^+) 979.

Methyl 5,6-biso-(2-anthroyl)-7-o-(9-anthroyl)-2,3-o-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranoside (34) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.96 (s, 1 H), 8.55 (s, 1 H), 8.50 (s, 1 H), 8.25 (s, 1 H), 8.24 (s, 1H), 8.14–7.88 (m, 9 H), 7.64 (s, 1 H), 7.60–7.45 (m, 8 H), 7.20–7.10 (m, 4 H), 6.16–6.15 (m, 1H, H-6), 6.05 (dd, J = 9.32 Hz, J = 2.36 Hz, 1 H, H-5), 5.37 (dd, J = 12.39 Hz, J = 3.82 Hz, 1 H, H-7), 4.94 (s, 1 H, H-1), 4.91 (dd, J = 12.32 Hz, J = 4.39 Hz, 1 H, H-7), 4.72 (dd, J = 5.74 Hz, J = 3.66 Hz, 1 H, H-3), 4.51 (d, J = 5.84 Hz, 1 H, H-2), 4.41 (dd, J = 9.33 Hz, J = 3.63 Hz, 1H, H-4), 3.16 (s, 3 H, 1-OCH_3), 1.47 (s, 3 H, -CCH_3), 1.18 (s, 3 H, -CCH_3); MS/CI (M^+) 877.

Methyl 5,6-bis-o-(p-methoxycinnamoyl)-7-o-(9-anthroyl)-2,3-o-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranoside (35) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.31 (s, 1 H), 8.06 (d, J = 8.78 Hz, 2 H), 7.85 (d, J = 7.85 Hz, 2 H), 7.68 (d, J = 15.83 Hz, 1 H), 7.47–7.36 (m, 8 H), 7.18 (d, J = 8.52 Hz, 1 H), 6.88 (d, J = 8.69 Hz, 2 H), 6.82 (d, J = 8.69, 2 H), 6.41 (d, J = 15.98 Hz, 1 H), 6.02 (d, J = 15.88 Hz, 1 H), 5.88 (bs, 1H, H-6), 5.69 (dd, J = 9.67 Hz, J = 2.46 Hz, 1 H, H-5), 5.10 (dd, J = 12.14 Hz, J = 3.7 Hz, 1 H, H-7), 4.89 (s, 1H, H-1), 4.73 (dd, J = 11.92 Hz, J = 6.26 Hz, 1 H, H-3), 4.66 (dd, J = 9.31 Hz, J = 3.68 Hz, 1 H, H-7), 4.50 (d, J = 6.05 Hz, 1 H, H-2), 4.18 (dd, J = 9.09 Hz, J = 3.54 Hz, 1H, H-4), 3.83 (s, 3 H, 1-OCH_3), 1.39 (s, 3 H, -CCH_3), 1.21 (s, 3 H, -CCH_3).

Methyl 6,7-bis-o-(2-anthroyl)-2,3-o-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranoside (36) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.89 (s, 1 H), 8.73 (s, 1 H), 8.54 (s, 1 H), 8.43 (s, 1 H), 8.40 (s, 1 H), 8.38 (s, 1 H), 8.04–7.95 (m, 8 H), 7.94–7.45 (m, 4 H), 5.97–5.95 (m, 1 H, H-6), 4.93 (dd, J = 11.55 Hz, J = 4.96 Hz, 1 H, H-7), 4.92 (s, 1 H, H-1), 4.85 (m, 1 H, H-3), 4.80 (dd, J = 11.62 Hz, J = 7.65 Hz, 1 H, H-7), 4.53 (d, J = 5.94 Hz, 1 H, H-2), 4.44–4.43 (m, 1 H, H-5), 4.06 (dd, J = 8.28 Hz, J = 3.88 Hz, 1 H, H-4), 3.20 (s, 3 H, 1-OCH_3), 3.10 (bs, 1 H, 5-OH), 1.26 (s, 3 H, -CCH_3), 1.23 (s, 3 H, -CCH_3); MS/FAB (M^+) 672.

Methyl 5,7-bis-o-(2-anthroyl)-2,3-o-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranoside (37) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.44 (s, 1 H), 8.34 (s, 1 H), 8.23 (s, 1 H), 8.03 (s, 1 H), 7.99 (s, 1 H), 7.70 (s, 1 H), 7.49–7.29 (m, 12 H), 5.72–5.71 (m, 1 H, H-5), 5.33 (bs, 1 H), 4.97 (s, 1 H, H-1), 4.83–4.81 (m, 1 H), 4.60 (bs, 1 H), 4.55 (d, J = 5.89 Hz, 1 H, H-2), 4.52 (bs, 1 H), 4.42 (bs, 1 H), 3.34 (s, 3 H, 1-OCH_3), 1.30 (s, 3 H, -CCH_3), 1.23 (s, 3 H, -CCH_3); MS/FAB (M^+) 672.

Methyl 6-o-(2-anthroyl)-7-o-(9-anthroyl)-2,3-o-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranoside (38) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.88 (s, 1 H), 8.55 (s, 1 H), 8.48 (s, 1 H), 8.46 (s, 1 H), 8.05–7.95 (m, 10 H), 7.54–7.29 (m, 4 H), 5.95–5.92 (m, 1H, H-6), 5.10–5.08 (m, 2 H, H-7), 4.90 (m, 1 H, H-1), 4.82 (dd, J = 5.80 Hz, J = 3.64 Hz, 1 H, H-3), 4.51 (d, J = 5.95 Hz, 1 H, H-2), 4.43–4.40 (m, 1 H, H-5), 4.03 (dd, J = 7.8 Hz, J = 3.69 Hz, 1H, H-4), 3.20 (s, 3 H, 1-OCH_3), 3.10 (bs, 1 H, 5-OH), 1.39 (s, 3 H, -CCH_3), 1.22 (s, 3 H, -CCH_3); MS/FAB (M^+) 672.

Methyl 5,6-bis-o-(2-anthroyl)-7-o-(9-anthroyl)-2,3-o-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranoside (40) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.79 (s, 1 H), 8.60 (s, 1 H), 8.45 (s, 1 H), 8.39 (s, 1 H), 8.37 (s, 1 H), 8.30 (s, 1 H), 8.20 (s, 1 H), 8.02–7.78 (m, 9 H), 7.53–7.46 (m, 4 H), 7.26–7.19 (m, 5 H), 7.16–7.00 (m, 2 H), 6.28–6.24 (m, 1 H), 6.06–6.04 (m, 1 H), 5.34–5.24 (m, 2 H), 5.08 (s, 1 H), 4.85–4.82 (m, 1 H), 4.62–4.58 (m, 2 H), 3.48 (s, 3 H), 1.50 (s, 3 H), 1.23 (s, 3 H); MS/CI (M^+) 876.

Methyl 5,6-bis-o-(p-methoxycinnamoyl)-7-o-(9-anthroyl)-2,3-o-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranoside (41) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.50 (s, 1 H), 8.08 (d, J = 8.58 Hz, 1 H), 7.99 (d, J = 5.53 Hz, 1 H), 7.68 (d, J = 16.14 Hz, 1 H), 7.62 (d, J = 15.98 Hz, 1 H), 7.47–7.41 (m, 4 H), 6.92–6.86 (m, 4 H), 6.39 (d, J = 16.04 Hz, 1 H), 6.27 (d, J = 16.06, 1 H), 6.01–5.99 (m, 1H, H-6), 5.72 (dd, J = 8.77 Hz, J = 2.34 Hz, 1 H, H-5), 5.13 (dd, J = 11.96 Hz, J = 8.29 Hz, 1 H, H-7), 5.04 (s, 1H, H-1), 5.00 (d, J = 3.51 Hz, 1 H, H-4), 4.79 (dd, J = 5.40 Hz, J = 3.52 Hz, 1 H, H-3), 4.59 (d, J = 5.86 Hz, 1 H, H-2) 4.37 (dd, J = 9.08 Hz, J = 4.04 Hz, 1 H, H-7), 3.86 (s, 3 H, 1-OCH_3), 1.48 (s, 3 H, -CCH_3), 1.30 (s, 3 H, -CCH_3); MS/CI (M^+) 788.

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