CHIRALITY 11:707–721 (1999)

Theoretical and Experimental CD of Conformationally Flexible Complex Molecules—Application to Ouabain Pentanaphthoate and Analogs

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ABSTRACT Theoretical calculation of circular dichroic exciton-coupled spectra of ouabain 1,19,2′,3′,4′-pentanaphthoate and its analogs was executed by a combination of conformational analysis with molecular mechanics and quantum-chemical calculation. Most of the calculated CD spectra show good agreement with the corresponding experimental data, which suggests that the method may be generalized for applications to other conformationally flexible natural products. The CD calculation was then used to evaluate the plausibility of "fortuitous CD cancellation," which was observed in the microscale naphthoylation study of hypothalamic inhibitory factor, a presumed ouabain isomer from bovine hypothalamus. *Chirality 11:707–721, 1999.*

KEY WORDS: exciton coupled CD; theoretical calculations of CD; accidental CD cancellation; 2-naphthoate chromophore; glycosylation; hypothalamic inhibitory factor; cardiac glycosides

The circular dichroic (CD) exciton chirality method has been widely used to determine the absolute configuration/conformation of a wide variety of organic molecules.[1,2] It is based on the through-space chiral interaction between the electric transition moments of two or more chromophores, which preexist in the molecule[3] or, in most cases, are introduced by derivatization of functional groups (-OH,-NH_2, etc.). In conformationally rigid molecules, the intramolecular chromophoric interactions give rise to a distinct exciton-coupled CD spectrum from which the absolute configuration can be determined nonempirically.[1] On the other hand, when the chromophore attachment sites are conformationally flexible, as in acyclic compounds, the CD spectrum arises from the contributions of all present conformers and becomes difficult to interpret. In several cases of conformationally flexible bioactive compounds, e.g., polyols derived from sugars,[4] bacteriohopanoid,[5] sphingolipids,[6] and heptopyranosides/heptofuranosides,[7] all possible stereochemical isomers were synthesized, and differentiation of isomers was achieved by bichromophoric derivatization, followed by CD measurements in polar and nonpolar solvent systems. Such CD libraries are now serving as references for stereochemical assignments of new compounds belonging to a specific type. Although this approach expands the applicability of the exciton chirality method to conformationally flexible molecules, it is time-consuming and laborious to synthesize all possible isomers and then to find appropriate CD conditions that maximally differentiate the isomers.

An alternative approach for structural analysis of conformationally flexible molecules is to compare experimental data with theoretically calculated CD of conformers within a certain energy range. Theoretical CD calculation has already been employed successfully for the structural assignment of many conformationally flexible molecules,[8–18] where the calculations were performed by a combination of conformational analysis and quantum-chemical calculation. Conformational analysis gives the plausible conformers and their relative energies, while quantum-chemical calculation yields the CD of all conformers: the theoretical CD spectrum is the weighted average of the obtained CD spectra. Reliability of CD calculation largely depends on the accuracy of the conformational analysis step, which has been carried out by semi-empirical calculations (AM1)[8–15] or with molecular mechanics calculations (MM2).[16–18] Our previous studies on vinblastin[16,17] and naphthoates of ouabagenin[18] demonstrated that a combination of a Monte Carlo conformational search with the MM2 force field on MacroModel V5.5[19,20] and π-electron SCF-CI-DV MO method[1,2] could provide calculated CD spectra that are in good agreement with the experimental data. These studies demonstrated that conformational analysis coupled with

Contract grant sponsor: NIH; Contract grant numbers: HL 52282, GM 34509.
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Received for publication 16 March 1999; Accepted 29 May 1999

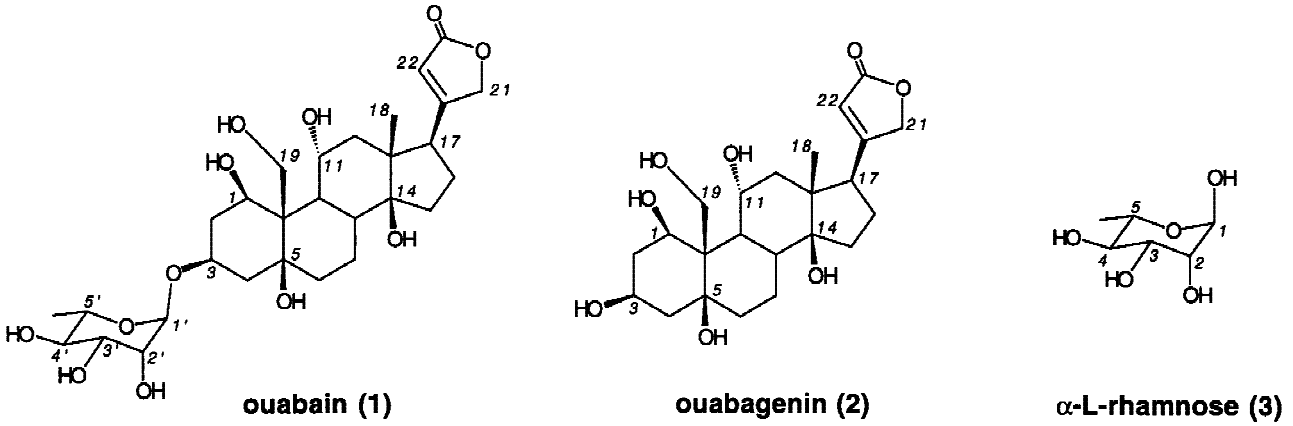


Fig. 1.

molecular mechanics calculations, a protocol which is applicable to a wider range of compounds than semiempirical calculations, can be sufficiently accurate to be used in theoretical CD calculations, provided the parameters and restrictions in the conformational analysis are appropriately adjusted for the compounds of interest.

This article presents our CD calculations of ouabain 1,19,2′,3′,4′-pentanaphthoate and its analogs, the results of which are compared with experimental data of synthetic analogs. It was our interest to check whether calculations could be applied to such complex systems, consisting of five interacting chromophores attached to a framework of substantially larger flexibility than those previously studied.[16–18] In addition, the current study aims at addressing a controversial issue which was encountered during our past nanogram-scale structural studies on the hypothalamic inhibitory factor (HIF), a sodium pump inhibitor from bovine hypothalamus.[21,22]

Structural studies on HIF have long been hindered by the extreme paucity of the sample material. The available structural information only indicates its close resemblance to ouabain **1,** a cardiac glycoside of plant origin (Fig. 1). In fact, many microscale spectroscopic and chemical analyses, such as LC/MS, LC/MS of aglycone, sugar analyses, etc., did not detect any difference between HIF and ouabain.[21] On the other hand, our nanogram-scale naphthoylation studies on HIF yielded a product with no CD couplet ("zero-CD" profile), which was in contrast to the clear positive CD of ouabain 1,19,2′,3′,4′-pentanaphthoate, the major product from ouabain naphthoylation.[21,22] This "zero-CD" compound was tentatively assigned as "HIF pentanaphthoate," despite the somewhat ambiguous FABMS.[21] HIF has since been considered a subtle structural isomer of ouabain.

However, it has been questionable whether a pentanaphthoate of a compound with many stereogenic centers could show the "zero-CD" profile at the concentration used for the CD measurement of "HIF pentanaphthoate." From the UV spectrum of "HIF pentanaphthoate," which was not presented in the published article,[22] its concentration estimated from the UV absorption at 234 nm was found to have been about 5×10^{-7} M. Since the background noise level in the CD measurement was less than 0.2 millidegrees (mdeg), the CD of "HIF pentanaphthoate" at this concentration should have been below the noise level. Therefore, CD amplitude of "HIF pentanaphthoate" at this concentration should have been below the noise level. Therefore, CD amplitude of "HIF pentanaphthoate" can be roughly estimated to be between −10 and +10: estimation can be done with the equation $\theta/33 = \Delta\varepsilon \times c \times 1$ (θ: ellipticity angle (degree); 1: cell length (cm); c: concentration (M)). Such weak CD of "HIF pentanaphthoate" was totally unexpected when compared to the strong positive CD couplet of ouabain 1,19,2′,3′,4′-pentanaphthoate with an amplitude of +472 (see Figs. 2, 4a). All the exciton couplets arising from the five interacting naphthoates in "HIF pentanaphthoate" had to be canceled out in order to show such weak CD. It is already experimentally and theoretically proven that the split CD curve of a compound containing three or more identical or different coupled chromophores can be fairly well represented by summation of each interacting basis pair.[18,23] Thus, in the case of "HIF pentanaphthoate" the presence of five interacting naphthoates would lead to summation of 10 basis pairs, which have to mostly cancel out to exhibit the "zero-CD" profile.

The plausibility of such accidental CD cancellation in "HIF pentanaphthoate" was subjected to reexamination by theoretical CD calculations of the possible isomers of ouabain. The available structural information[21] led to the possibility that HIF could be a sugar positional isomer of ouabain. This possibility was in accordance with the indistinguishable LC/MS profiles between HIF-genin and ouabagenin 2 (Fig. 1) and could explain the observation of both HIF and ouabain having the same α-L-rhamnoside moiety 3 (Fig. 1).[21] On the other hand, it was questionable, if not impossible, whether both ouabain and one of the sugar positional isomers could really share the same LC/MS profile as seen in the previous study.[21] However, isomers other than sugar positional isomers, such as aglycone configurational or constitutional isomers, etc., seemed even less likely to reproduce the LC/MS profiles of both HIF and its genin, which left the sugar positional isomers as the only group of compounds for the current study. Many of the pentanaphthoates of sugar positional isomers

can be readily prepared by synthesis and used to verify the results of CD calculations. This study, therefore, provides a good opportunity to further examine the accuracy of the CD calculation protocol, which has so far not been tested with compounds with such a high degree of conformational flexibility.

In the following, the CD calculation protocol is first evaluated by comparison between theoretical and experimental data of the synthetically accessible compounds. This is followed by a check to examine the possibility of a fortuitous CD cancellation in "HIF pentanaphthoate."

METHODS OF CALCULATION

Molecular Geometry

Conformers of each compound and their relative energies were obtained with the Monte Carlo conformational search on MacroModel V5.5.[19,20] Under the assumption that the lactone had a negligible effect on the exciton coupling between the naphthoate chromophores, this moiety was omitted from calculations to save computer time. In a pilot study, the molecular modeling studies on ouabain pentanaphthoate and its analogs were carried out by using various force fields, such as MM2 and AMBER, without any restrictions. However, since CD calculations based on the geometry generated by such a nonrestricted conformational search did not reproduce the experimental CD curves, the following restrictions were made in the conformational analysis:

1) All free primary, secondary hydroxyl groups and the C-5 tertiary hydroxyl group were "protected" as acetates before calculation since the hydrogen bonding effect may not be correctly estimated by the approach employed in this study. Moreover, as the acetates were experimentally readily available, if necessary their CD could be measured for direct comparison with calculated data. The employment of acetate protection was also well-justified by the observed close similarity between experimental CD amplitudes of the pentanaphthoates with unprotected and protected hydroxyls (Fig. 2), namely, 3-Rha/1,19 PN (pentanaphthoate) **4** (+472/+399), 3-Rha/1,11-PN **5** (+667/+661), and 3-Rha/11,19-PN **6** (+243/+261).

Furthermore, the calculated CD of pentanaphthoates with protected hydroxyls (Fig. 2) showed close agreement with the experimental data of corresponding pentanaphthoates, with either protected or free hydroxyls. In contrast, the calculated CD of pentanaphthoates with free hydroxyls differed significantly from the experimental data, most likely due to inaccurate conformational search, which could have overestimated the hydrogen bonding effect.

2) Following conformational search, the conformers in which the naphthalene ring and the carbonyl of all naphthoates were set as being coplanar were submitted to further local energy minimization with the MM2 force field (in MacroModel V5.5) in $CHCl_3$ to improve the convergence of the conformational search. The coplanar conformation restriction was made based on the following considerations. First, X-ray crystal data of ouabagenin 1,3,19-trisnaphthoate show that the conformation of all three naphthoates are coplanar (unpublished results of J. Gougoutas); and second, if some pentanaphthoates significantly deviate from coplanarity, the decreased orbital overlap between the aromatic ring and the carbonyl should give rise to a blue shift in the ^1B_b naphthoate transitions compared with that of simple cyclohexyl 2-naphthoate. However, experimental data do not show such differences, e.g., the λmax of cyclohexyl 2-naphthoate is at 234 nm, while those of pentanaphthoates 3-Rha/1,19-PN **4,** 3-Rha/1,11-PN **5,** 3-Rha/11,19-PN **6,** 1-Rha/3,19-PN **16,** and 11-Rha/3,19-PN **23** are also at 233–234 nm.

Finally, all minimized structures have been submitted to local minimization until they converged. For conformers with close energies, their root mean square deviation (RMSD) was obtained in order to see if they belonged to the same conformational family. RMSD values (<0.6 Å) have been used to assign minimized structures to conformational families.[24] In the current study, minimized structures were considered to be in the same conformational family if RMSD values were <0.5 Å. All remaining conformers within the 3 kcal/mol energy range from the lowest energy conformer (3 kcal/mol cutoff) were used for subsequent estimation of the CD.

π-Electron SCF-CI-DV MO Method

The CD and UV of each conformer were calculated according to the π-electron SCF-CI-DV MO method,[1,2] in which only the π–π* transitions of naphthoate chromophores were taken into account for the MO calculation (the CD computation program was provided by N. Harada, Sendai). The configuration interaction between 1,050 singly excited states of lower energy were included in the calculations. The atomic orbital parameters are as follows: for sp^2 carbons, Z(C) = 1.0, Wc = –11.16 eV, (rr|rr)(C) = 11.13 eV, β(C-C, 1.388A) = –2.42 eV, <▽>(C-C, 1.388A) = 4.701 × 10^7 cm^{-1}; for ether oxygens (sp^3), Z(O) = 2.0, W(O) = –33.00 eV, (rr|rr)(O) = 21.53 eV, β(C-O) = –2.11eV, <aP>(C-O) = 6.00 × 10^7 cm^{-1}; for carbonyl oxygens (sp^2), Z(O) = 2.0, W(O) = –17.28 eV., (rr|rr)(O) = 14.58 eV, β(C-O) = –2.54 eV, <▽>(C-O) = 5.00 × 10^7 cm^{-1}. The electric repulsion integral (rr|ss) was estimated by the Nishimoto-Mataga equation.[1,25] The component CD and UV curves were approximated by Gaussian distribution, and the standard deviation Δσ values 2,250 cm^{-1} were taken from the half value of 1/e bandwidth of the observed UV spectrum of cyclohexyl-2-naphthoate.

The theoretical CD spectrum of each isomer was the weighted average of the calculated CD spectra of all conformers within the 3 kcal/mol cutoff range. The weighting was carried out with the Boltzmann distribution, which had been obtained from the relative energies of the conformers.

RESULTS AND DISCUSSION

The two main objectives of the current study were to examine the accuracy of the CD calculation protocol with ouabain 1,19,2′,3′,4′-pentanaphthoate and its synthetically available analogs, and then to check the plausibility of a fortuitous CD cancellation in "HIF pentanaphthoate" using the theoretical and experimental CD for pentanaphthoates of ouabain sugar (α-L-rhamnose) positional isomers. Some considerations prior to CD calculations allowed us to re-

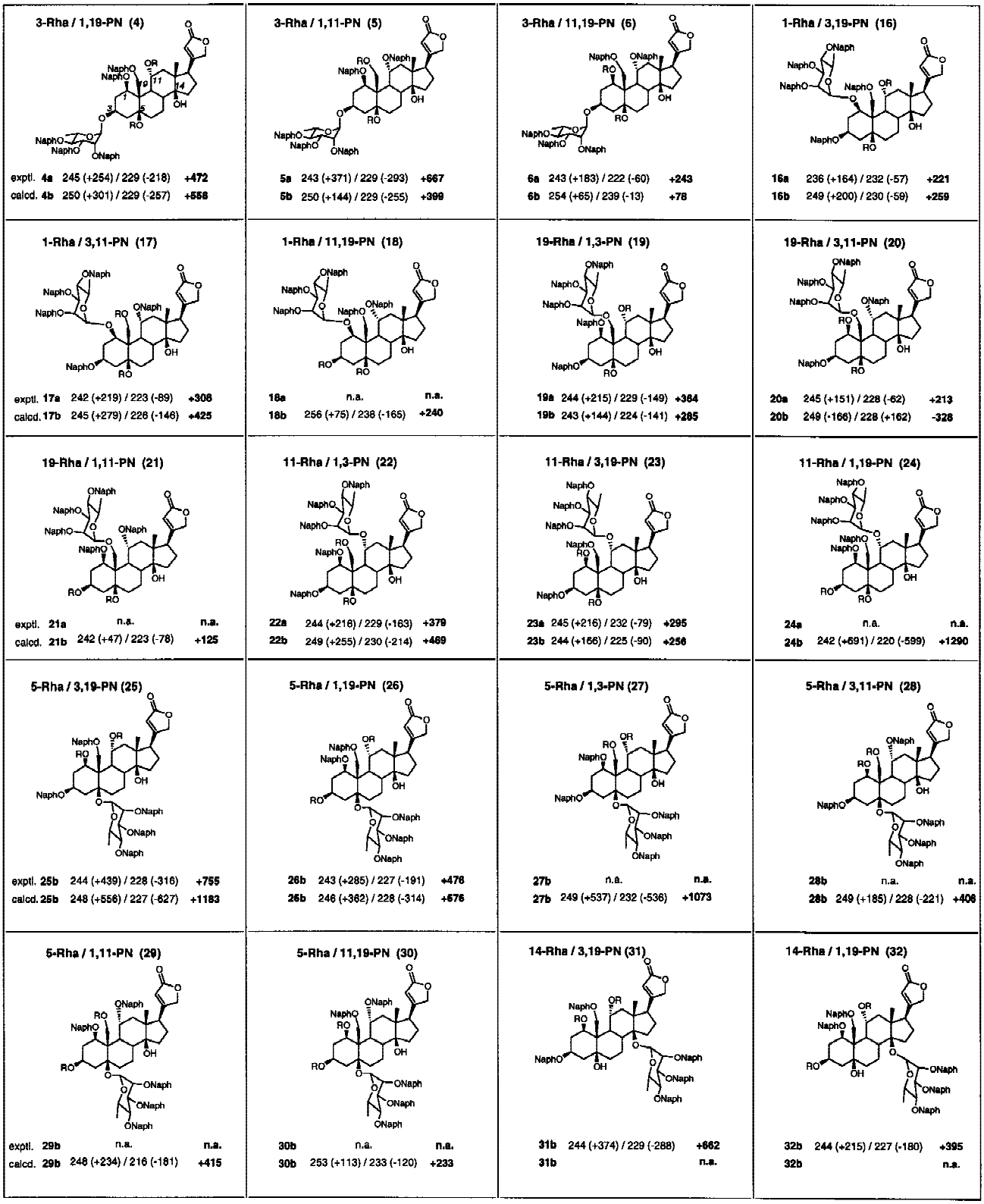
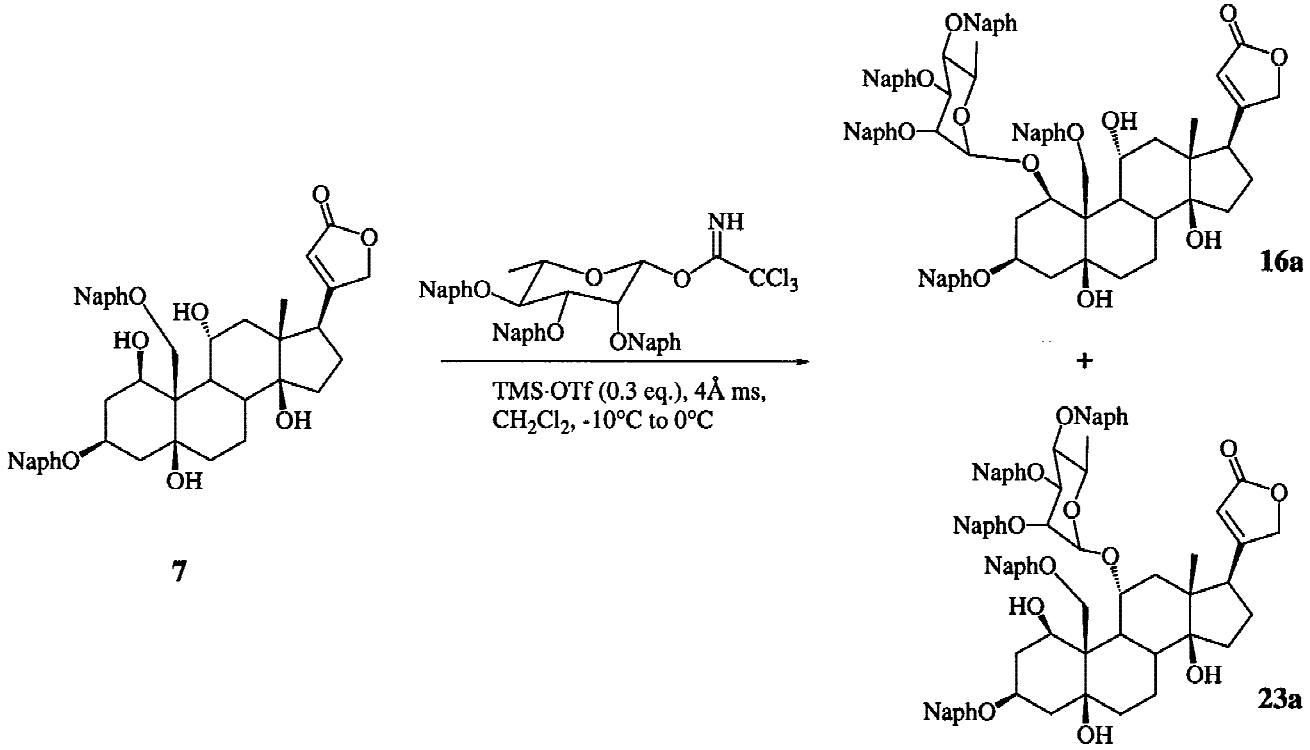


Fig. 2. Exptl. (in MeCN) and calcd. CD data of ouabain pentanaphthoate and analogs. a: R = H; b: R = Ac. Top and bottom lines denote exptl. and calcd. CD data, respectively. A values, given in bold, correspond to the weighted average CD of all conformers within 3 kcal/mol energy range; n.a. denotes that data are not available.



Scheme 1. Glycosylation of ouabagenin bisnaphthoate (7) with trisnaphthoylated L-rhamnose trichloroimidate to give a mixture of pentanaphthoates **16a** and **23a.**

duce the number of relevant pentanaphthoates to be calculated. While there are only five ouabain sugar positional isomers, i.e., 1-, 5-, 11-, 14-, and 19-α-L-rhamnosides of ouabagenin, the number of their pentanaphthoates jumps to 280, since each isomer has eight free hydroxyl groups: there are ${}_8C_5 = 56$ permutations to make a pentanaphthoate for each isomer. Most of them can be excluded from the study for the following reasons. The possibility of a 14-rhamnoside appeared unlikely since a free 14-β-hydroxyl is necessary for biological activities of cardenolides.[26] In addition, our earlier FABMS analysis of "HIF pentanaphthoate" indicated that three naphthoyl groups were on the sugar moiety,[21] which further reduced the number of possible isomers. The remaining two naphthoyl groups were assumed to be on the free primary or secondary hydroxyl groups on the genin moiety since naphthoylation does not usually occur at the sterically hindered tertiary hydroxyl groups under the condition used for HIF naphthoylation. The resulting 15 possible isomers together with the three pentanaphthoates of ouabain were thus submitted to CD calculations (see Fig. 2).

Preparation of Ouabain Pentanaphthoate Analogs

It was attempted to prepare as many pentanaphthoates as possible in order to check the calculated results with experimental data. Naphthoylation of ouabain yielded three pentanaphthoates, 1,19,2′,3′,4′-pentanaphthoate **4a** (3-Rha/1,19-PN), 1,11,2′,3′,4′-pentanaphthoate **5a** (3-Rha/1,11-PN), and 11,19,2′,3′,4′-pentanaphthoate **6a** (3-Rha/11,19-PN). It is noted that the obtained compounds have three naphthoates on the sugar moiety, which is in accordance with our assumption discussed above. As for the pentanaphthoates of sugar positional isomers, it was first attempted to synthesize α-rhamnosides which had rhamnose on one of the hydroxyls other than the 3-OH, and then to naphthoylate these α-rhamnosides under conditions used for HIF. Several glycosylation methods were tested for the coupling of the protected rhamnose moiety onto selectively protected ouabagenin: the examined methods included glycosyl bromide and chloride; phenyl sulfoxide sugar in the presence of Tf_2O; 1-O-acyl (1-acetyl, naphthoyl, 2-pyridine carboxyl) sugar; and trichloroimidate-mediated glycosylation.[27–29] The trichloroimidate-mediated glycosylation was found most suited because of the almost instantaneous nature of the reaction, the satisfactory yield, the absence of isomerization and/or dehydration reactions, and the high α/β ratio (important because α-rhamnosides were the targets). However, this route for the synthesis of sugar positional isomers had to be abandoned since various attempts to remove the acetate-protecting groups resulted in either no reaction or decomposition.

Since it was assumed that of the five naphthoyl groups three were on the rhamnose moiety, the pentanaphthoates could be assembled by coupling rhamnose trisnaphthoate with bisnaphthoates of ouabagenin (Scheme 1). Four bisnaphthoates of ouabagenin **7** (3,19-bisnaphthoate),[18] **8** (1,3-bisnaphthoate),[18] **9** (1,19-bisnaphthoate),[18] and **10** (3,11-bisnaphthoate), and two bisnaphthoate bisacetates **11** (the bisacetate of **7**) and **12** (the bisacetate of **9**) were available for the glycosylation reaction (Fig. 3). The structural assignment of these compounds was based on ^1H and COSY spectra. Although both H-1 and H-3 appear as broad

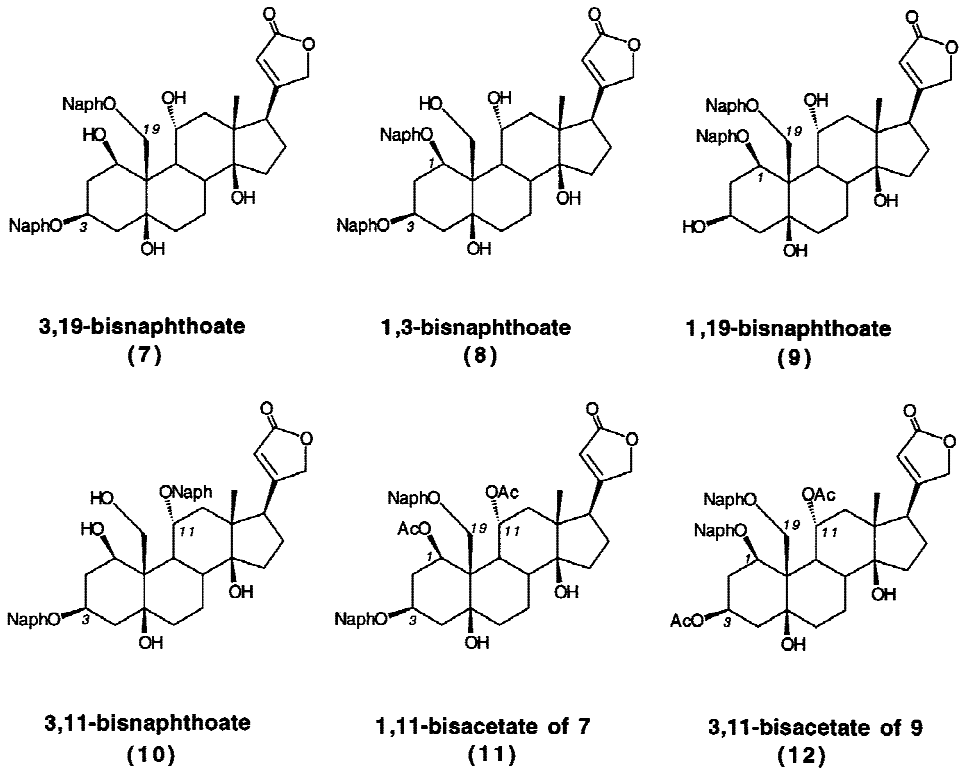


Fig. 3. Structures of some bisnaphthoates of ouabage-nin.

singlets in ^{1}H spectra, they could be distinguished from each other by COSY, because H-1 coupled with only H-2, while H-3 coupled with H-2 and with H-4. The assignment was also confirmed by selectively synthesizing 3,11-bisnaphthoate of ouabagenin from 1,19-acetonide of ouabagenin.

A total of ten α-L-rhamnoside positional isomers of ouabain 1,19,2′,3′,4′-pentanaphthoates were prepared by the coupling of 2,3,4-tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloroacetimidate **15** to the appropriately derivatized ouabagenins in the presence of 0.3 equivalents of TMS·OTf and 4 Å molecular sieves. Glycosylation occurred predominantly at the free primary and/or secondary hydroxyl groups on the bisnaphthoylated ouabagenins, **7, 8,** and **10,** through which the following isomers were obtained: **16a** (1-α-L-rhamnoside 3,19,2′,3′, 4′-pentanaphthoate; abbreviated to "1-Rha/3,19-PN") and **17a** (1-Rha/3,11-PN), **19a** (19-Rha/1,3-PN), and **20a** (19-Rha/3,11-PN), **22a** (11-Rha/1,3-PN) and **23a** (11-Rha/3,19-PN) (see structures in Fig. 2). On the other hand, glycosylation at the tertiary hydroxyl groups of **11** and **12** gave **25b** (5-Rha/3,19-PN) and **31b** (14-Rha/3,19-PN), **26b** (5-Rha/1,19-PN) and **32b** (14-Rha/1,19-PN), respectively. The rhamnoside linkage position was determined by HMBC and/or by ROESY. For example, the HMBC spectrum of 1-rhamnoside **17a** (1-Rha/3,11-PN) showed a strong cross-peak between the H-1′ and C-1, while the ROESY spectrum showed a cross-peak between H-1 and H-1′. For 19-rhamnoside **20a,** two ROESY cross-peaks between the H-1′ and the two protons of H-19 were observed.

The glycosylation reaction provided α-isomer as the major product, which was isolated by HPLC purification of the crude mixture; the α/β ratio was ca. 5/1 as estimated by NMR. The rationale for the preference of the α-isomer over the β-isomer could be attributed to the following: 1) β-imidates usually produce α-glycosides as the major anomer, and vice versa[28]; 2) the anomeric effect favors α-glycosides; 3) since the 2-OH in rhamnose is axial, the α-glycoside with 1,2-trans periplanar substituents is sterically favored over the β-glycoside. The stereochemistry at the anomeric center was confirmed by NOE studies. The β-rhamnosides with axial H-1′, H-3′, H-5′ show ROESY cross-peaks between H-1′/H-3′ and H-1′/H-5′, whereas for α-rhamnosides no cross-peaks were detected between H-1′/H-3′ and H-1′/H-5′.

Following the purification by normal phase HPLC, the α-glycosides were submitted to UV/CD measurements in acetonitrile.

CD Calculation of Pentanaphthoate Derivative of Ouabain and Its Analogs

In general, theoretically calculated exciton split CD spectra are sensitive to even small changes in molecular geometry when the changes affect the orientation of the interacting electric transition moments. The asymmetric 2-naphthoate chromophore, which was used in the current study, makes our calculations more challenging, since the chromophore can adopt both *s-cis* and *s-trans* conformations, which are energetically very close to each other.[18] In the case of cyclohexyl-mono-2-naphthoate, the *s-cis* conformer was found only 0.03 kcal/mol higher than the *s-trans* conformer by Macromodel studies. The two conform-

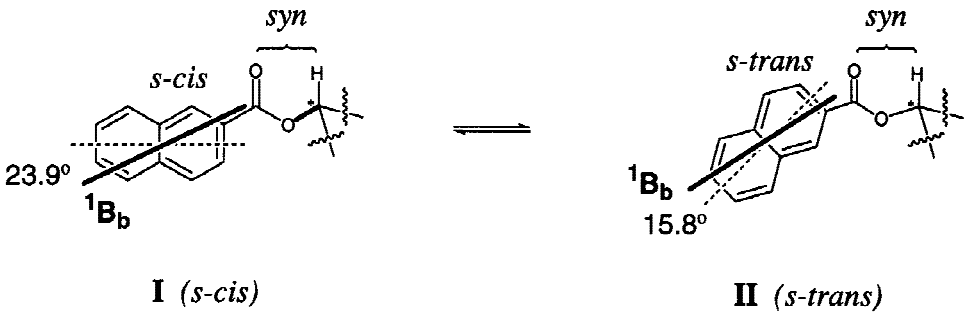


Fig. 4. *s-cis* and *s-trans* conformations of 2-naphthoate chromophore, polarization of ^1B_b transition and *syn* orientation of C(O)-/H-C* in the most preferred ester conformation.

ers showed small differences in the position, intensity, and orientation of ^1B_b band: the ^1B_b of the *s-cis* conformation is calculated to be rotated 23.9° from the longitudinal (orientation of ^1B_b transition of naphthalene), while the ^1B_b of the *s-trans* conformation is rotated by 15.8° (Fig. 4). The small differences between the *s-cis* and *s-trans* conformations seen in the mono-naphthoate derivative, however, could affect the current CD calculations, in which the spatial orientations of the five naphthoate chromophores need to be accurately estimated. Therefore, the naphthoate conformation was not restricted during the conformational search, so that both *s-cis* and *s-trans* conformations are considered: in fact, the conformational search revealed that each conformer has a different *s-cis/s-trans* ratio (for example, see Fig. 5).

Table 1 summarizes the conformational distribution profiles of all 18 analogs, which were obtained from the sequential calculation steps; i.e., 1) conformational search, 2) corrections for naphthoyl planarity, 3) local energy minimizations, 4) elimination of duplicated conformers by RMSD analysis, and 5) selection of the conformers within the 3 kcal/mol range from the lowest energy conformer. For example, 28 conformers were found within the 3 kcal/mol range in the conformational analysis of **17b** (1-Rha/3,11-PN). Of these 28 conformers, 17 were within 2 kcal/mol range and six conformers had energies within 1 kcal/mole range from the lowest energy conformer. These conformers differed mainly by ratios of *s-cis/s-trans* naphthoate chromophores. As shown in Figure 5, the lowest energy conformer has all *s-trans* 2-naphthoates, whereas one of the higher energy conformers within 1 kcal/mol shows *s-cis* at C-3, C-3′ and C-4′ and *s-trans* at C-11, and C-2′.

Since by all analogs the conformational population within 3 kcal/mol range represent almost 100% Boltzmann distribution, the CD calculations by the π-electron SCF-CI-DV MO method[1,2] have been performed on conformers within this range only. The theoretical CD spectrum of each analog was obtained as a weighted average of the calculated CD spectra for the conformers in the 3 kcal/mol

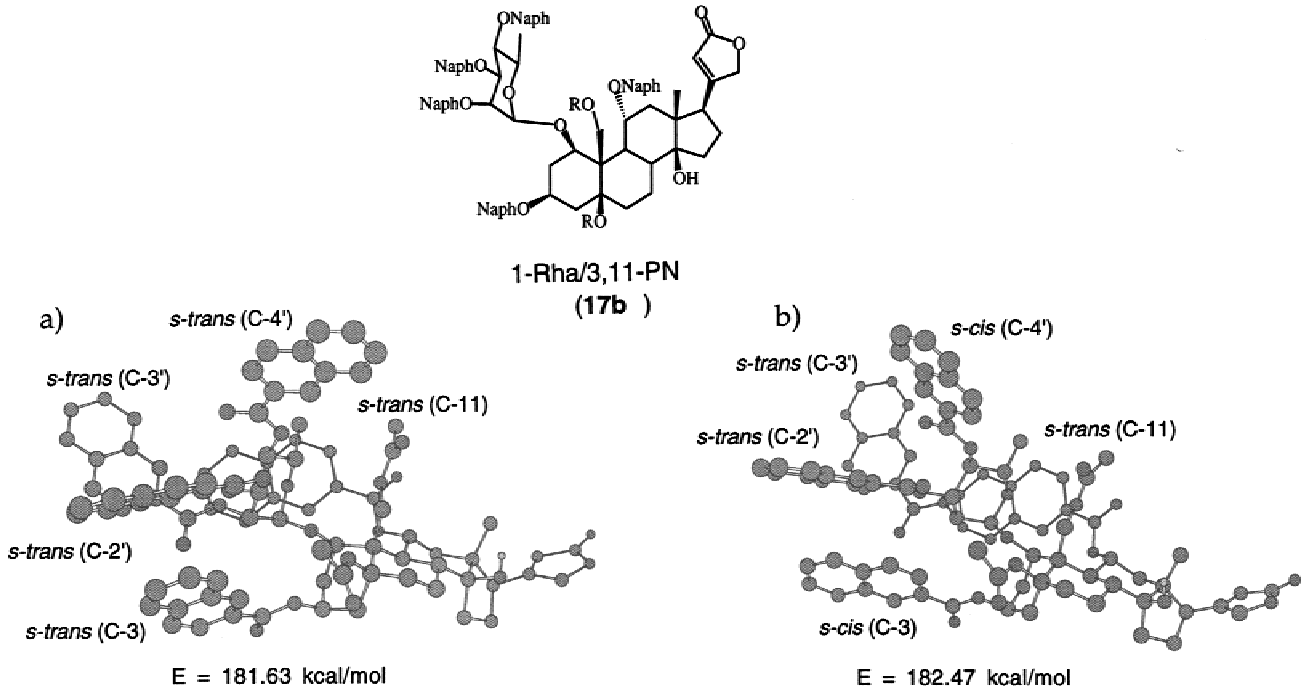


Fig. 5. (a) The lowest energy conformer of 1-Rha/3,11-PN **(17b);** (b) one of the five other conformers within 1kcal/mol range; *s-cis* and *s-trans* denote the conformations of 2-naphthoate chromophores.

TABLE 1. Conformational distributions (number of conformers and their population in %) within 1 kcal/mol, 2 kcal/mol and 3 kcal/mol energy ranges for ouabain pentanaphthoate and its analogs

	Number of conformers and their population in %		
Compound	Within 1 kcal/mol	Within 2 kcal/mol	Within 3 kcal/mol
4b	12 (89.3)	22 (99.3)	27 (100)
5b	6 (74.5)	9 (95.5)	14 (100)
6b	12 (87.2)	22 (99.2)	25 (100)
16b	7 (88.2)	14 (99.6)	16 (100)
17b	6 (75.4)	17 (95.5)	28 (100)
18b	11 (89.3)	16 (96.9)	26 (100)
19b	10 (75.0)	23 (99.0)	26 (100)
20b	8 (88.9)	14 (97.5)	20 (100)
21b	4 (88.3)	6 (97.6)	10 (100)
22b	14 (78.4)	31 (99.7)	33 (100)
23b	6 (65.3)	27 (97.1)	34 (100)
24b	4 (97.0)	5 (98.7)	8 (100)
25b	4 (89.4)	7 (97.7)	10 (100)
26b	5 (86.9)	11 (99.3)	12 (100)
27b	3 (92.6)	4 (93.8)	15 (100)
28b	4 (64.6)	16 (93.7)	26 (100)
29b	5 (91.4)	8 (96.9)	17 (100)
30b	8 (81.7)	17 (99.0)	20 (100)

range. The weighting was carried out according to the Boltzmann energy distribution. In the case of ouabain 1,19,2′,3′,4′-pentanaphthoate **4b** (3-Rha/1,19-PN), the 27 conformers within the 3 kcal/mol range gave rise to its theoretical CD curve {(250 nm ($\Delta\varepsilon$ +301)/229 nm ($\Delta\varepsilon$ −257), A = +558}, which was in excellent agreement with the experimentally obtained CD curve {245 nm ($\Delta\varepsilon$ +254)/229 nm ($\Delta\varepsilon$ −218), A = +472} (Fig. 6). Although the agreement between the theoretical and the experimental CD curves of **4b** was good even when only the 1 kcal/mol range was taken into account for the calculation (Table 2), it is dangerous to use such a small energy range for CD calculation since by chance the remaining part, which usually represents 10 to 30% of the total conformer population (Table 1), could contain conformers with a very strong CD couplet. For example, the CD amplitude (*A*-value) of **17b** (1-Rha/3,11-PN) is much larger than the experimental value of +308 when only the 1 kcal/mol range was taken into account (Table 3). The difference between calculated and experimental data became smaller when the wider ranges of energy, 2 and 3 kcal/mol, were employed for the calculation (Fig. 7). The 3 kcal/mol range, therefore, assures that almost the entire conformer population is taken into account for the CD calculation.

The theoretical CD spectra of 11 compounds can be directly compared with the corresponding experimental data for the evaluation of our CD calculation protocol (Fig. 2). It is noted that the conformational analysis with the molecular mechanics is the inevitable source of calculation error, whereas the π-electron SCF-CI-DV MO calculation could also cause some additional error. Considering the many possible sources of error, our CD calculation protocol attained satisfactory results in most cases, i.e., **4b, 5b, 16b, 17b, 19b, 22b, 23b, 25b,** and **26b.** On the other hand,

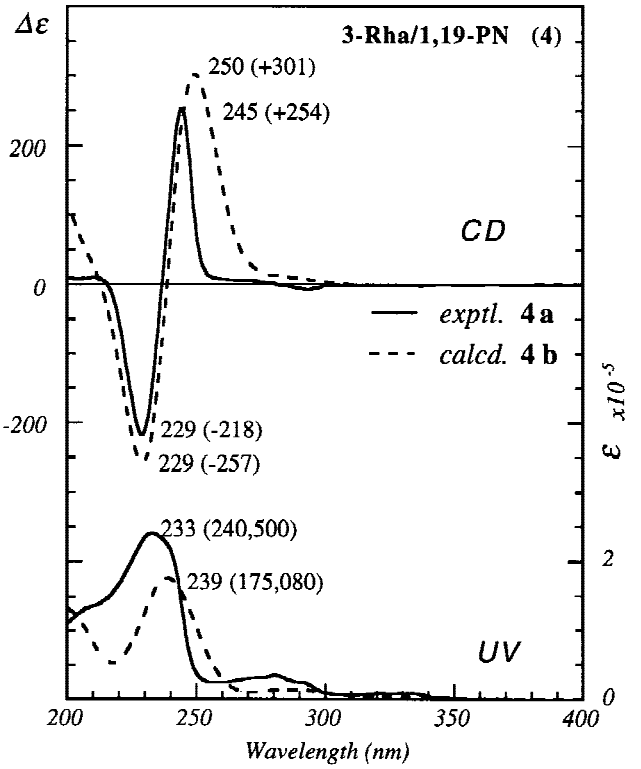


Fig. 6. The UV/CD spectra of ouabain pentanaphthoate **(4). 4a:** exptl. (in MeCN, solid line); **4b:** calcd. (dashed line).

the calculated CD spectra of **6b** and **20b** exemplify the limitation of our current CD calculation protocol.

The calculated CD couplet of **6b** (3-Rha/11,19-PN), 254 nm ($\Delta\varepsilon$ +65)/239 nm ($\Delta\varepsilon$ −13), A = +78, is much weaker than the experimental one, 243 nm ($\Delta\varepsilon$ +183)/222 nm ($\Delta\varepsilon$ −60), A = +243. The discrepancy appears to stem from the conformational flexibility of the C-19 naphthoate group, which can freely rotate because of the absence of bulky substituents around this moiety. Most of the low energy conformers of **6b** have the C-19 naphthoate pointing toward the area between C-1 acetate and C-11 naphthoate, whereas one conformer, with energy of 0.59 kcal/mol higher than the lowest, has C-19 naphthoate pointing toward the C-3 rhamnoside moiety. The latter conformer shows a CD curve that is close to the experimental data, namely, *A*-value of +237 (data not included in Table 2), indicating that our calculation protocol on the conformational search step is not accurate enough to correctly estimate the conformational profile of this freely rotating moiety.

The CD calculation of **20** (19-Rha/3,11-PN) presents a formidable task for our current protocol. In this case, not only does the flexible C-19 carry a large trisnaphthoylated rhamnose moiety, but it also lacks steric strain from neighboring substituents that could restrict rotation around the C-10/C-19 bond. Because of this conformational freedom of the 19-rhamnoside moiety, the conformational analysis of **20** is obviously beyond the capability of our current calculation protocol. The calculated CD shows a negative couplet, which is opposite to the experimental positive CD

TABLE 2. Comparison of calculated UV (λ_{max}(nm), ε) and CD (λ_{ex}(nm), Δε and A) data within different energy ranges for 3-Rha/1,19-PN (4b)

Energy range of conformers	Percentage*	UV, λ_{max}(nm), ε	CD, λ_{ex}(nm), Δε	A
1 kcal/mol	89.3%	239 (175,070)	250 (+304)/229 (–257)	+561
2 kcal/mol	99.3%	239 (175,100)	250 (+302)/229 (–257)	+559
3 kcal/mol	100.0%	239 (175,080)	250 (+301)/229 (–257)	+558

*It was assumed that the conformers within the 3 kcal/mol range had 100% probability in the solution.

curve. In order to clarify possible reasons for this discrepancy, we performed a conformational search and CD calculations on 19-Rha/3,11-PN **20a** (all free hydroxyls unprotected) in two versions: (i) all naphthoate groups were kept coplanar; (ii) with no constraint regarding naphthoate coplanarity. In (i) the CD calculation of the lowest energy conformer again showed a negative and even more intense exciton couplet with A = –897. In (ii) the conformational search disclosed that the torsion angles between carbonyl groups and the naphthalene planes of the lowest energy conformer varied from 7° to 25°. In this case, the calculated negative CD amplitude A = –1,370 was not only opposite in sign but dramatically different from the experimental one A = +213. These results emphasize the difficulties encountered in the CD calculations of compounds with large flexibility. It is also worth noting that the calculated CD of another 19-rhamnoside, 19-Rha/1,3-PN **19b,** where the presence of the bulky C-1 naphthoyl group restricts the rotation of the 19-rhamnoside, is in good agreement with the experimental value.

The current results indicate that our calculation protocol can predict the CD of ouabain pentanaphthoate analogs with fair accuracy unless the compound contains the C-19 chromophoric group (naphthoate or trisnaphthoylated rhamnose), orientation of which is not restricted by steric interactions with neighboring bulky substituents. Accordingly, the structures of 1-Rha/11,19-PN **18,** 19-Rha/1,11-PN **21,** 11-rha/1,19-PN **24,** 5-Rha/1,3-PN **27,** 5-Rha/3,11-PN **28,** 5-Rha/1,11-PN **29,** and 5-Rha-11,19-PN **30** suggest that although these pentanaphthoates were not obtained by synthesis, the calculated values should closely represent experimental values. Namely, it can be anticipated that the calculated CD of **27, 28,** and **29** are fairly accurate since they lack chromophoric groups at 19-OH. As for **18, 21, 24,** and **30,** which have a chromophoric group at C-19, the calculation results are still considered reliable since they all have a bulky substitution at either C-1 or C-5 that restricts the rotation of the C-19 chromophore. Although there are obvious limitations in the described conformational analysis and in the CD calculation protocol, the current results are encouraging because of the possible development of more versatile CD calculations for conformationally flexible molecules.

Possibility of the Fortuitous CD Cancellation

The strongly UV absorbing 2-naphthoate chromophore (ε 58,000 at 234 nm) was originally employed in the structural analysis of HIF to enhance the presumed subtle structural differences between HIF and ouabain, which might not be detected with the weaker chromophores such as benzoates. Even though the sample concentration was dilute (estimated to be ca. 5×10^{-7} M) for CD measurement, "HIF pentanaphthoate" was expected to give some CD couplet since most of the five naphthoates (except 19-) were directly attached to stereogenic centers, including those on the rhamnoside. The CD amplitude of each ouabain pentanaphthoate isomer can be approximated by the summation of 10 pairs ($_5C_2 = 10$) of bisnaphthoate couplings (pairwise additivity rule[18]). Since the naphthoate pairs within the rhamnoside moiety give rise to a strong positive CD (cf. rhamnose 2,3,4-trisnaphthoate shows +797), it is not hard to imagine that the statistical distribution of the CD amplitudes of all possible isomers, including sugar positional isomers, aglycone configurational isomers, alcohol positional isomers, etc., should be centered in the high positive region. Namely, the isomers that could exhibit such a weak CD (roughly between –10 to +10), if they do exist at all, must be an extremely small fraction of all possible isomers. The accidental CD cancellation of all 10 pairwise exciton-coupled contributions is, therefore, a severe structural requirement that could exclude most of the ouabain isomers from the search for HIF. Since it was technically impossible to synthesize all possible isomers of ouabain pentanaphthoate, the theoretical CD calculations were the only feasible approach to search for a possible HIF candidate. If the candidate is found by this CD calculation

TABLE 3. Comparison of calculated UV (λ_{max}(nm), ε) and CD (λ_{ex}(nm), Δε and A) data within different energy ranges for 1-Rha/3,11-PN (17b)

Energy range of conformers	Percentage*	UV, λ_{max}(nm), ε	CD, λ_{ex}(nm), Δε	A
1 kcal/mol	75.4%	238 (222,300)	245 (+311)/226 (–166)	+477
2 kcal/mol	95.5%	238 (220,100)	245 (+278)/226 (–147)	+425
3 kcal/mol	100.0%	238 (220,020)	245 (+279)/226 (–146)	+425

*It was assumed that the conformers within the 3 kcal/mol range had 100% probability in the solution.

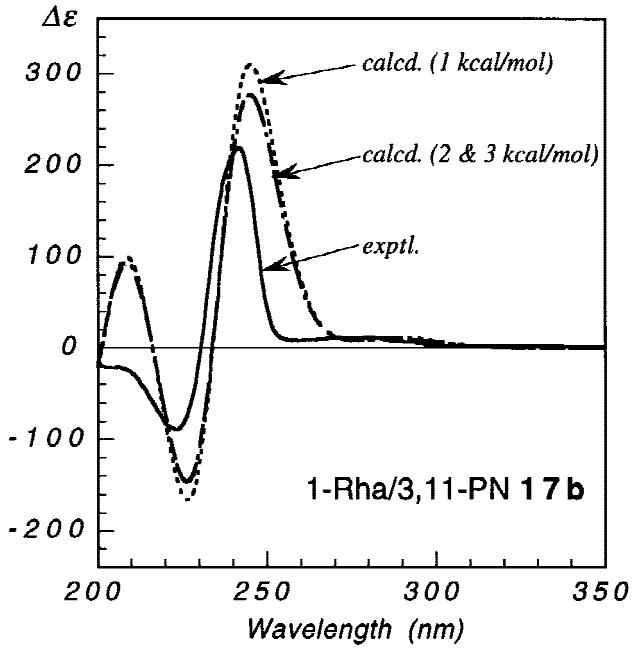


Fig. 7. CD spectra of 1-Rha/3,11-PN **(17). 17a:** exptl. (in MeCN, solid line); **17b:** calcd. within 1 kcal/mol (dashed line), within 2 kcal/mol and 3 kcal/mol the CD spectra are overlapped completely (broken line).

method, it can be synthesized and checked as to whether it reproduces the LC/MS profiles of HIF and its genin, which were identical to ouabain and ouabagenin, respectively.[21]

No conceivable candidate for HIF was found in the current theoretical and experimental CD study on the sugar positional isomers of ouabain. The CD results were further corroborated by the reversed phase HPLC analysis of the synthesized isomers. While the HIF product had shorter retention time than ouabain 1,19,2′,3′,4′-pentanaphthoate,[21,22] all of the tested isomers were found to have much longer retention time than ouabain 1,19,2′,3′,4′-pentanaphthoate under the same HPLC conditions (Table 4). Isomers other than sugar positional isomers, such as aglycone configurational isomers, alcohol positional isomers, etc., were excluded from the current study since they did not seem to satisfy the severe criteria for HIF, i.e., identical LC/MS profiles with ouabain (for the rhamnoside) and ouabagenin (for the aglycone) and the fortuitous CD cancellation (for the pentanaphthoate). Nullification of CD couplets resulting from interactions between five naphthoate groups, which was the possible explanation for the "zero-CD" profile of "HIF pentanaphthoate,"[21,22] could not be supported from the current CD analysis of the ouabain sugar positional isomers, the only group of possible isomers that satisfies the requirement for the aglycone, which has to be identical to ouabagenin by LC/MS. The present results rather made us doubt the past assignment of the "zero-CD" compound to "HIF pentanaphthoate,"[21] which was questioned previously because of the ambiguous FABMS result.[30]

Very recently, this long-standing enigma of the "zero-CD" product was resolved in a totally unexpected manner.[31] Subsequent to the CD study presented in this article, 3 μg of HIF was purified from bovine hypothalamus tissue. ^{1}H-NMR analysis led us to identify the purified material as ouabain-borate complexes, which did not give ouabain pentanaphthoate in our naphthoylation conditions due to this unexpected cyclic borate protection of the hydroxyl groups. Borate complexation occurred after HPLC purification in borosilicate glasswares because of the minuscule quantity of the purified material. In addition, reexamination of the past 200 nanogram-scale naphthoylation studies revealed that the "zero-CD" product turned out to be glycerol trisnaphthoate, which had most likely been caused by an incidental contamination of glycerol in the original HIF sample. Therefore, the conspicuous "zero-CD" product as well as the absence of ouabain pentanaphthoate had misled the past structural study on HIF. In retrospect, the CD study presented in this article gave us the first sign of this "unexpected" conclusion of HIF structural analysis. Our current experience underscores the importance of further development of theoretical CD calculation methods for the microscale structural analysis of natural products that are available only in minuscule quantities.

CONCLUSIONS

Ouabain pentanaphthoates and analogs are conformationally flexible complex molecules. The through-space coupling of the five naphthoate groups with intense UV absorptions give rise to well-defined exciton-coupled CD spectra. The CD spectra of the ouabain pentanaphthoate and analogs have been theoretically calculated and compared with the CD data of 11 synthetic naphthoates. Although there are some exceptions, generally good agreement has been found between calculated and experimental data. These calculated and experimental CD data negate the fortuitous CD nullification of through-space chromophoric interactions, a rationalization advanced to account for the zero CD of "HIF pentanaphthoate"; in turn, this discrepancy resulted in reexamination of the nature of HIF.[31] The preliminary theoretical CD calculations of complex systems may be generalized for applications to other conformationally flexible natural products.

TABLE 4. Retention times of ouabain pentanaphthoate and its analogs under reversed phase HPLC condition

No.	Compound	Retention time (min)[a]
4a	3-Rha/1,19-PN	11.4
5a	3-Rha/1,11-PN	13.9
6a	3-Rha/11,19-PN	17.8
16a	1-Rha/3,19-PN	17.3
17a	1-Rha/3,11-PN	15.8
19a	19-Rha/1,3-PN	25.5
20a	19-Rha/3,11-PN	15.7
22a	11-Rha/1,3-PN	16.3
23a	11-Rha/3,19-PN	12.2
25b[b]	5-Rha/3,19-PN	25.0
26b[b]	5-Rha/1,19-PN	23.2

[a]HPLC condition: gradient elution from 82% MeCN in H_2O.
[b]All free hydroxyl groups in **25b** and **26b,** except 14-OH, are acetylated (see Fig. 2).

MATERIALS AND METHODS

All materials were purchased from Aldrich (Milwaukee, WI) except ouabain and ouabagenin, which were purchased from Sigma (St. Louis, MO). Methylene chloride was distilled from calcium hydride under nitrogen. Acetonitrile was Aldrich anhydrous grade for reactions and spectrophotometric grade for UV and CD analysis. ^{1}H NMR spectra were recorded on a Bruker DMX500 spectrometer. Analytical and preparative TLC was run on precoated silica-gel plates (Analtech, 20 cm × 20 cm, 250, 500, or 1,000 microns). The final purification of the analogs used the following HPLC equipment and method: Waters 600E multisolvent delivery system with a Waters 600 controller, a Water 996 photodiode array detector and Millenium 2010 software for data processing. The normal phase HPLC column was 250 × 10 mm I.D., S-5 µm, 120 Å). HPLC analysis of the retention times of the analogs was performed with a Perkin-Elmer (Norwalk, CT) Model series 4 liquid chromatography terminal coupled with a Hewlett-Packard (Corvallis, OR) 1046 programmable fluorescence detector (detected at λ_{ex} = 234 nm, λ_{emi} = 360 nm) with a Vydac C_{18} column (4.6 × 250 mm, 10 µm). UV spectra were taken on a Perkin-Elmer Lambda 6 model. The concentration of each synthetic analog was estimated by using the molecular extinction coefficient of ouabain 1,19,2′,3′,4′-pentanaphthoate; ε = 240,500 (λ_{max} = 233 nm). CD spectra were measured on a Jasco J-720 spectropolarimeter. All calculations were carried out with Indigo Silicon Graphics 3D workstation at the Department of Chemistry, Columbia University.

CHEMISTRY

Naphthoylation of Ouabain (1)

A mixture of ouabain (10 mg, 13.74 µmol), 1-(2-naphthoyl)imidazole (55 mg, 0.25 mmol) and DBU (31 µL, 0.21 mmol) in 2.3 mL of MeCN was stirred at room temperature overnight. It was then quenched with water and extracted with CH_2Cl_2. The crude mixture contained three pentanaphthoates: 1β,19-Bis-2-naphthoyloxy-3β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,11α,14β-trishydroxy-5β-card-20(22)-enolide **(4a);** 1β, 11α-Bis-2-naphthoyloxy-3β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,14β,19-trishydroxy-5β-card-20(22)-enolide **(5a);** 11α,19-Bis-2-naphthoyloxy-3β-{(2,3,4-tris-O-2-naphthoyl-α-L- rhamnopyranosyl)oxy}-1β,5,14β-trishydroxy-5β-card-20(22)-enolide **(6a),** which were separated and purified (**4a:5a:6a** = 485:1:63) by silica gel plate (500 µm, 20 cm × 20 cm) chromatography (30:1 $CHCl_3$/MeOH).

1β,19-Bis-2-naphthoyloxy-3β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl) oxy}-5,11α,14β-trishydroxy-5β-card-20(22)-enolide (3-Rha/1,19-PN) (4a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.61 (s, 1H), 8.54 (s, 1H), 8.30 (s, 1H), 8.16-7.04 (m, 31H), 7.32 (s, 1H, H-1), 5.85 (s, 1H, H-22), 5.77 (s, 1H, H-2′), 5.66 (t, J = 10.1 Hz, 1H, H-4′), 5.42 (dd, J = 10.1 Hz, J = 3.1 Hz, 1H, H-3′), 5.34 (s, 1H, H-1′), 5.33 (d, J = 12.1 Hz, 1H, H-19), 5.26 (d, J = 12.1 Hz, 1H, H-19), 4.90 (d, J = 11.0 Hz, 1H, H-21), 4.75 (d, J = 10.8 Hz, 1H, H-21), 4.49 (s, 1H, H-3), 4.15-4.03 (m, 2H, H-5′, H-11), 1.18 (d, J = 5.9 Hz, 3H, H-6′), 0.86 (s, 3H, H-18). MS (FAB pos.) $(M + H + Na)^+$ m/z 1378.

1β,11α-Bis-2-naphthoyloxy-3β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,14β,19-trishydroxy-5β-card-20(22)-e nolide (3-Rha/1,11-PN) (5a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.66 (s, 1H), 8.56 (s, 1H), 8.29 (s, 1H), 8.12-7.00 (m, 31H), 6.51 (s, 1H, H-1), 6.12-6.06 (m, 1H, H-11), 5.89 (s, 1H, H-22), 5.76 (s, 1H, H-2′), 5.65 (t, J = 10.0 Hz, 1H, H-4′), 5.45 (dd, J = 10.2 Hz, J = 3.2 Hz, 1H, H-3′), 5.36 (s, 1H, H-1′), 4.85 (d, J = 19.2 Hz, 1H, H-21), 4.78 (d, J = 18.5 Hz, 1H, H-21), 4.57 (d, J = 12.3 Hz 1H, H-19), 4.54 (s, 1H, H-3), 4.33 (d, J = 11.5 Hz, 1H, H-19), 4.09-4.06 (m, 1H, H-5′), 1.21 (d, J = 6.2 Hz, 3H, H-6′), 1.13 (s, 3H, H-18). MS (FAB pos.) $(M + H + Na)^+$ m/z 1378.

11α,19-Bis-2-naphthoyloxy-3β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-1β,5,14β-trishydroxy-5β-card-20(22)-enolide (3-Rha/11,19-PN) (6a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.50 (s, 1H), 8.49 (s, 1H), 8.31 (s, 1H), 8.18 (s, 1H), 8.15-7.30 (m, 30H), 5.92 (dd, J = 10.2 Hz, J = 3.1 Hz, 1H, H-3′), 5.87-5.83 (m, 2H, H-22, H-4′), 5.80 (s, 1H, H-2′), 5.68-5.60 (m, 1H, H-11), 5.35 (s, 1H, H-1′), 5.34 (d, J = 12.0 Hz, 1H, H-19), 5.26 (d, J = 12.4 Hz, 1H, H-19), 5.09 (s, 1H, H-1), 4.82 (d, J = 18.0 Hz, 1H, H-21), 4.74 (d, J = 18.7 Hz, 1H, H-21), 4.51-4.47 (m, 2H, H-3′, H-5′), 2.80 (t, J = 6.3 Hz, 1H, H-17), 1.40 (d, J = 6.2 Hz, 3H, H-6′), 1.07 (s, 3H, H-18). MS (FAB pos.) $(M + H + Na)^+$ m/z 1378.

Bisnaphthoylation of ouabagenin (2)

A mixture of oubagenin (25 mg, 56.95 µmol), 1-(2-naphthoyl)imidazole (27 mg, 0.12 mmol) and DBU (35 µL, 0.23 mmol) in 4 mL of MeCN was stirred at room temperature for 10 min. It was then quenched with water and extracted with CH_2Cl_2. The crude mixture contained four bisnaphthoates: 3β,19-Bis-2-naphthoyloxy-1β,5,11α,14β-tetrahydroxy-5β-card-20(22)-enolide (7), 1β,3β-Bis-2-naphthoyloxy-5,11α,14β,19-tetrahydroxy-5β-card-20(22)-enolide **(8),** 1β,19-Bis-2-naphthoyloxy-3β,5,11α,14β-tetrahydroxy-5β-card-20(22)-enolide **(9),** and 3β,11α-Bis-2-naphthoyloxy-3β,5,14β,19-tetrahydroxy-5β-card-20(22)-enolide **(10),** which were separated and purified (**7:8:9:10** = 18:1:21:6) by silica gel plate (1,000 µm, 20 cm × 20 cm) chromatography (39:1 $CHCl_3$/MeOH).

3β,11α-Bis-2-naphthoyloxy-3β,5,14β,19-tetrahydroxy-5β-card-20(22)-enolide (10). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.49 (s, 1H), 7.99-7.79 (m, 3H), 7.59-7.48 (m, 5H), 5.88 (s, 1H, H-22), 5.69 (bs, 1H, H-3), 5.56 (m, 1H, H-11), 4.91 (bs, 1H, H-1), 4.86 (d, J = 17.9 Hz, 1H, H-21), 4.76 (d, J = 18.2 Hz, 1H, H-21), 4.63 (d, J = 11.3 Hz, 1H, H-19), 4.20 (d, J = 11.4 Hz, 1H, H-19), 3.81 (s, 1H), 2.80-1.37 (m, 17H), 1.08 (s, 3H, H-18). MS (CI neg.) $(M-H)^-$ m/z 745.

General Acetylation Procedure

A mixture of 3β,19-Bis-2-naphthoyloxy-1β,5,11α,14β-tetrahydroxy-5β-card-20(22)-enolide **7** (3.3 mg, 4.42 µmol),

Ac_2O (0.01 mL, 0.11 mmol) and anhydrous pyridine (0.04mL, 0.49 mmol) was stirred overnight. It was then quenched with water, extracted with CH_2Cl_2, and the organic phase was washed three times with saturated aqueous solution of $CuSO_4$. Evaporation of the solvent and purification by silica gel plate (500 µm, 20 cm × 20 cm) chromatography (39:1 $CHCl_3$/MeOH) afforded 3β,19-Bis-2-naphthoyloxy-1β,11α-bisacetoxy-5,14β-tetrahydroxy-5β-card-20(22)-enolide (11). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.47 (s, 1H), 8.07-7.82 (m, 8H), 7.61-7.50 (m, 4H), 6.22 (s, 1H, H-1), 5.87 (s, 1H, H-22), 5.67 (s, 1H, H-3), 5.23-5.19 (m, 2H, H-19, H-11), 5.05 (d, J = 12.5 Hz, 1H, H-19), 4.85 (dd, J = 18.0 Hz, J = 1.3 Hz, 1H, H-21), 4.73 (dd, J = 17.9 Hz, J = 1.5 Hz, 1H, H-21), 4.26 (s, 1H), 2.73-2.71 (m, 1H), 2.54–2.39 (m, 3H), 2.21–1.01 (m, 13H), 2.07 (s, 3H, CH_3CO), 1.71 (s, 3H, CH_3CO), 0.94 (s, 3H, H-18). MS (FAB pos.) $(M + H)^+$ m/z 831.

1β,19-Bis-2-naphthoyloxy-3β,11α-bisacetoxy-5,14β-tetrahydroxy-5β-card-20(22)-enolide (12)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.35 (s, 1H), 7.95–7.73 (m, 8H), 7.57–7.48 (m, 4H), 6.48 (s, 1H, H-1), 5.86 (s, 1H, H-22), 5.42 (s, 1H, H-3), 5.29–5.14 (m, 3H, H-19, H-19, H-11), 4.85 (dd, J = 17.8 Hz, J = 1.7 Hz, 1H, H-21), 4.73 (dd, J = 17.9 Hz, J = 1.6 Hz, 1H, H-21), 4.37 (s, 1H), 2.73–2.71 (m, 1H), 2.58–2.54 (m, 1H), 2.45–2.35 (m, 2H), 2.13 (s, 3H, CH_3CO), 2.20–1.34 (m, 13H), 1.60 (s, 3H, CH_3CO), 0.94 (s, 3H, H-18). MS (FAB pos.) $(M + H)^+$ m/z 831.

1,2,3,4-Tetrakis-O-2-naphthoyl-β-L-rhamnopyranose (13)

A mixture of rhamnose (400 mg, 2.19 mmol) and 2-naphthoyl chloride (2,600 mg, 13.64 mmol) in anhydrous pyridine (6 mL, 74.18 mmol) was stirred at room temperature overnight. It was then quenched with water and extracted with CH_2Cl_2. The organic layer was washed three times with a saturated aqueous solution of $CuSO_4$ and dried with Na_2SO_4. Column chromatography (solvent: 10% EtOAc in hexanes) of the crude mixture afforded the pure product as a white solid (457 mg, 54% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.56 (s, 1H), 8.48 (s, 1H), 8.38 (s, 1H), 8.30–7.30 (m, 24H), 6.51 (s, 1H, H-1), 6.26 (d, J = 1.76 Hz, 1H, H-2), 5.97–5.90 (2H, H-3, H-4), 4.29–4.19 (m, 1H, H-5) 1.59 (d, J = 6.2 Hz, 3H, H-6). MS (FAB pos.) $(M + K)^+$ m/z 820.

2,3,4-Tris-O-2-naphthoyl-α,β-L-rhamnopyranose (14)

A mixture of **13** (457 mg, 0.59 mmol), methyl alcohol (0.19 mL mg, 4.58 mmol) and AcBr (0.4 mL, 5.35 mmol) in CH_2Cl_2 (4 mL) was stirred at room temperature for 1 h. It was then quenched with water and extracted with Et_2O. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was evaporated to give the crude bromide, which was treated with acetone (4 mL), a drop of water, and Ag_2CO_3 (600 mg, 2.18 mmol). Removal of Ag_2CO_3 by gravity filtration and evaporation of the solvent afforded almost pure compound **14** as a fluffy white solid (98 mg, 27% yield for two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.55 (s, 1H), 8.35 (s, 1H), 8.15–7.30 (m, 18H), 6.07 (dd, J = 10.1 Hz, J = 3.4 Hz, 1H, H-3), 5.88–5.82 (2H, H-2, H-4), 5.55 (s, 1H, H-1), 4.62–4.51 (m, 1H, H-5), 1.45 (d, J = 6.2 Hz, 3H, H-6). MS (FAB pos.) $(M + Na)^+$ m/z 649.

2,3,4-Tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloroacetimidate (15)

A mixture of **14** (33 mg, 0.05 mmol) and K_2CO_3 (37 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was stirred at room temperature for 10 min, at which point CCl_3CN (0.029 mL, 0.29 mmol) was added and the resulting mixture was stirred for 3 h. The solvent was then evaporated and the crude product was directly chromatographed (solvent: 20% EtOAc in hexanes) to give pure compound **15** as a fluffy white solid (30 mg, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.69 (s, 1H), 8.55 (s, 1H), 8.34 (s, 1H), 8.15–7.30 (m, 18H), 6.60–6.20 (2H, H-2, H-3), 5.96 (t, J = 7.3 Hz, 1H, H-4), 4.55–4.48 (m, 1H, H-5) 1.50 (d, J = 6.2 Hz, 3H, H-6). MS (CI neg.) $(M - H)^-$ m/z 770.

Glycosylation of 3β,19-Bis-2-naphthoyloxy-1β,5,11α,14β-tetrahydroxy-5β-card-20(22)-enolide (7)

A mixture of 3,19-bis-O-2-naphthoyl-ouabagenin (7) (15 mg, 20.11 µmol) and 4 Å molecular sieves in CH_2Cl_2 (0.8 mL) was stirred for 10 min at room temperature and then cooled to −10°C. A solution of 2,3,4-tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloroacetimidate **15** (16 mg, 0.02 mmol) in CH_2Cl_2 (0.2 mL) was added and the mixture was stirred for an additional 10 min. TMS·OTf (0.001 µL, 5.53 nmol) diluted in CH_2Cl_2 (11 µL) was then added and the reaction was almost instantaneous, yielding rhamnosides **16a** and **23a.** The mixture was stirred for 15 min at −10°C and then warmed to 0°C over a 20-min period. It was then quenched with H_2O, extracted with CH_2Cl_2, and dried over anhydrous Na_2SO_4. The two regioisomers were separated by plate (500 µm, 20 cm × 20 cm) chromatography (silica gel, 19:1 $CHCl_3$/MeOH). **16a** (0.5 mg) and **23a** (10.2 mg).

3β,19-Bis-2-naphthoyloxy-1β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,11α,14β-trishydroxy-5β-card-20(22)-enolide (1-Rha/3,19-PN) (16a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.54 (s, 1H), 8.34 (s, 1H), 8.32 (s, 1H), 8.12–7.30 (m, 28H), 7.01–6.83 (m, 3H), 5.94 (s, 1H, H-3), 5.80–5.75 (m, 3H, H-22, H-2′, H-1), 5.54–5.48 (m, 2H, H-4′, H-19), 5.38–5.28 (m, 3H, H-3′, H-19, H-1′), 4.84 (d, J = 18.3 Hz, 1H, H-21), 4.4.67 (dd, J = 18.8 Hz, J = 1.4 Hz, 1H, H-21), 4.33–4.27 (m, 2H, H-11, H-5′), 2.90–2.87 (m, 1H), 2.72–2.54 (m, 3H), 2.18–1.42 (m, 13H), 0.99 (d, J = 6.2 Hz, 3H, H-6′), 0.67 (s, 3H, H-18). MS (FAB pos.) $(M + 2H)^+$ m/z 1356.

3β,19-Bis-2-naphthoyloxy-11α-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-1β,5,14β-trishydroxy-5β-card-20(22)-enolide (11-Rha/3,19-PN) (23a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.57 (s, 1H), 8.55 (s, 1H), 8.32 (s, 1H), 8.31 (s, 1H), 8.13–7.32 (m, 29H), 7.03 (t, 1H), 5.98 (s, 1H, H-22), 5.80 (s, 1H, H-3), 5.75 (d, J = 15.0 Hz, 1H, H-19), 5.73 (dd, J = 9.7 Hz, J = 3.9 Hz, 1H, H-3′), 5.64 (t, J = 9.8 Hz, 1H, H-4′), 5.40 (s, 1H, H-2′), 5.27 (s, 1H, H-1), 5.12 (d, J = 12.9 Hz, 1H, H-19), 4.97 (d, J

= 18.6 Hz, 1H, H-21), 4.83 (d, J = 18.0 Hz, 1H, H-21), 4.82 (s, 1H, H-1′) 4.61–4.58 (m, 1H, H-11), 4.08–4.02 (m, 1H, H-5′), 2.95–2.93 (m, 1H), 2.69–2.58 (m, 3H), 2.29–1.24 (m, 13H), 1.19 (d, J = 6.1 Hz, 3H, H-6′), 1.09 (s, 3H, H-18). MS (FAB pos.) $(M + 2H)^+$ m/z 1356.

Glycosylation of 3β,11α-Bis-2-naphthoyloxy-3β,5,14β,19-tetrahydroxy-5β- card-20(22)-enolide (10)

A mixture of 3β,11α-Bis-2-naphthoyloxy-3β,5,14β,19-tetrahydroxy-5β-card-20(22)-enolide **10** (21 mg, 28.15 μmol) and 4 Å molecular sieves in CH_2Cl_2 (1.2 mL) was stirred for 10 min at room temperature and then cooled to −10°C. A solution of 2,3,4-tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloracetimidate **15** (22 mg, 28.54 μmol) in CH_2Cl_2 (0.4 mL) was added and the mixture was stirred for an additional 10 min. TMS·OTf (0.002 μL, 11.05 nmol) diluted in CH_2Cl_2 (16 μL) was then added and the reaction was almost instantaneous, yielding rhamnosides **17a** and **20a.** The mixture was stirred for 15 min at −10°C and then warmed to 0°C over a 20-min period. It was then quenched with H_2O, extracted with CH_2Cl_2, and dried over Na_2SO_4. The two regioisomers were separated by plate (500 μm, 20 cm × 20 cm) chromatography (silica gel, 19:1 $CHCl_3$/MeOH). **17a** (6.4 mg) and **20a** (9.9 mg).

3β,11α-Bis-2-naphthoyloxy-1β-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,14β,19-trishydroxy-5β-card-20(22)-e nolide (1-Rha/3,11-PN) (17a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.48 (s, 1H), 8.42 (s, 1H), 8.19 (s, 1H), 7.92–6.86 (m, 31H), 5.90 (bs, 2H, H-11, H-22), 5.80 (bs, 1H, H-3), 5.61 (dd, J = 10.1 Hz, J = 3.3 Hz, 1H, H-3′), 5.32 (t, J = 10.1 Hz, 1H, H-4′), 5.20 (s, 1H, H-1), 5.13 (d, J = 2.0 Hz, 1H, H-2′), 4.89 (d, J = 18.1 Hz, 1H, H-21), 4.79 (d, J = 17.8 Hz, 1H, H-21), 4.73 (d, J = 11.4 Hz, 1H, H-19), 4.66 (s, 1H, H-1′), 4.52 (s, 1H), 4.37 (d, J = 11.9 Hz, 1H, H-19), 4.29–4.23 (m, 1H, H-5′), 2.92–1.19 (m, 17H), 1.60 (s, 3H, H-18), 0.96 (d, J = 6.2 Hz, 3H, H-6′). MS (FAB pos.) $(M + H)^+$ m/z 1355.

3β,11α-Bis-2-naphthoyloxy-19-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-1β,5,14β-trishydroxy-5β-card-20(22)-enolide (19-Rha/3,11-PN) (20a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.58 (s, 1H), 8.53 (s, 1H), 8.49 (s, 1H), 8.33 (s, 1H), 8.16–7.18 (m, 30H), 6.07 (dd, J = 10.2 Hz, J = 3.27 Hz, 1H, H-3′), 5.98–5.93 (m, 1H, H-11), 5.91 (s, 1H, H-22), 5.87–5.85 (m, 2H, H-2′, H-4′), 5.74 (s, 1H, H-3), 5.26 (d, J = 1.4 Hz, 1H, H-1′), 4.93 (d, J = 18.6 Hz, 1H, H-21), 4.86–4.83 (m, 2H, H-1, H-21), 4.54 (s, 2H, H-19), 4.46–4.41 (m, 1H, H-5′), 2.91–2.90 (m, 1H), 2.64–1.49 (m, 16H), 1.45 (d, J = 6.2 Hz, 3H, H-6′), 1.32 (s, 3H, H-18). MS (FAB pos.) $(M + H)^+$ m/z 1355.

Glycosylation of 1β,3β-Bis-2-naphthoyloxy-5,11α,14β,19-tetrahydroxy-5β-card-20(22)-enolide (8)

A mixture of 1β,3β-Bis-2-naphthoyloxy-5,11α,14β,19-tetrahydroxy-5β-card-20(22)-enolide **8** (8.7 mg, 11.66 μmol) and 4 Å molecular sieves in CH_2Cl_2 (0.4 mL) was stirred for 10 min at room temperature and then cooled to −10°C. A solution of 2,3,4-tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloroacetimidate **15** (9 mg, 11.67 μmol) in CH_2Cl_2 (0.2 mL) was added and the mixture was stirred for an additional 10 min. TMS·OTf (0.003 μL, 16.58 nmol) diluted in CH_2Cl_2 (13 μL) was then added and the reaction was almost instantaneous, yielding rhamnosides **19a** and **22a.** The mixture was stirred for 15 min at −10°C and then warmed to O°C over a 20–min period. It was then quenched with H_2O, extracted with CH_2Cl_2, and dried over Na_2SO_4. The two regioisomers were separated by plate (500 μm, 20 cm × 20 cm) chromatography (silica gel, 19:1 $CHCl_3$/MeOH). **19a** (1.4 mg) and **22a** (1.7 mg).

1β,3β-Bis-2-naphthoyloxy-19-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,11α,14β-trishydroxy-5β-card-20(22)-enolide (19-Rha/1,3-PN) (19a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.65 (s, 1H), 8.58 (s, 1H), 8.44 (s, 1H), 8.31–7.27 (m, 29H), 7.01–6.90 (m, 2H), 6.71 (s, 1H, H-1), 6.33 (dd, J = 10.1 Hz, J = 3.4 Hz, 1H, H-3′), 5.98 (t, J = 9.9 Hz, 1H, H-4′), 5.94 (s, 1H, H-22), 5.91 (s, 1H, H-2′), 5.84–5.80 (bs, 1H, H-3), 5.39 (s, 1H, H-1′), 4.97–4.89 (m, 2H, H-11, H-21), 4.82 (d, J = 18.1 Hz, 1H, H-21), 4.76 (d, J = 11.1 Hz, 1H, H-19), 4.64–4.61 (m, 1H, H-5′), 4.37 (d, J = 9.5 Hz, 1H, H-19), 3.24-3.21 (m, 1H), 2.97–2.94 (m, 1H), 2.84–2.80 (m, 1H), 2.66–2.61 (m, 1H), 2.30–1.53 (m, 13H), 1.50 (d, J = 6.1 Hz, 3H, H-6′), 0.98 (s, 3H, H-18). MS (FAB pos.) $(M + Na)^+$ m/z 1377.

1β,3β-Bis-2-naphthoyloxy-11α-{(2,3,4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5,14β,19-trishydroxy-5β-card-20(22)-e nolide (11-Rha/1,3-PN) (22a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.65 (s, 1H), 8.52 (s, 1H), 8.37 (s, 1H), 8.26 (s, 1H), 8.21–7.27 (m, 27H), 7.07–7.02 (m, 3H), 6.98 (s, 1H, H-1), 6.31 (dd, J = 10.2 Hz, J = 3.5 Hz, 1H, H-3′), 5.95–5.90 (m, 2H, H-4′, H-22), 5.89 (dd, J = 3.3 Hz, J = 1.2 Hz, 1H, H-2′), 5.83 (s, 1H, H-3), 5.41 (s, 1H, H-1′), 5.02–4.98 (m, 1H, H-11), 4.94 (d, J = 18.4 Hz, 1H, H-21), 4.81 (dd, J = 19.4 Hz, J = 1.5 Hz, 1H, H-21), 4.83–4.79 (m, 2H, H-19, H-5′), 4.45 (dd, J = 13.8 Hz, J = 9.7 Hz, 1H, H-19), 4.36 (s, 1H), 3.20–3.17 (m, 1H), 2.91–2.81 (m, 2H), 2.70–2.64 (m, 1H), 2.45–1.47 (m, 13H), 1.44 (d, J = 6.2 Hz, 3H, H-6′), 1.01 (s, 3H, H-18). MS (FAB pos.) (M + Na)$^+$ m/z 1377.

Glycosylation of 3β,19-Bis-2-naphthoyloxy-1β,11α-bisacetoxy-5,14β-tetrahydroxy-5β-card-20(22)-enolide (11)

A mixture of 3β,19-Bis-2-naphthoyloxy-1β,11α-bisacetoxy-5,14β-tetrahydroxy-5β-card-20(22)-enolide **11** (5 mg, 6.70 μmol) and 4 Å molecular sieves in CH_2Cl_2 (0.2 mL) was stirred for 10 min at room temperature and then cooled to −10°C. A solution of 2,3,4-tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloroacetimidate **15** (5 mg, 6.49 μmol) in CH_2Cl_2 (0.4 mL) was added and the mixture was stirred for an additional 10 min. TMS.OTf (0.002 μL, 11.05 nmol) diluted in CH_2Cl_2 (14 μL) was then added and the reaction was almost instantaneous. The mixture was stirred for 15 min at −10°C and then warmed to 0°C over a 20-min period. It was then quenched with H_2O, extracted with CH_2Cl_2, and dried over Na_2SO_4. Plate (500 μm, 20 cm

× 20 cm) chromatography (silica gel, 19:1 $CHCl_3$/MeOH) yielded rhamnosides **25b** (0.5 mg) and **31b** (4.0 mg).

3β,19-Bis-2-naphthoyloxy-1β,11α-bisacetoxy-5β-{(2,3,4 -tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-14β-hydroxy-5β-card-20(22)-enolide (5-Rha/3,19-PN) (25b). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.66 (s, 1H), 8.62 (s, 1H), 8.52 (s, 1H), 8.40 (s, 1H), 8.14–7.35 (m, 30H), 6.00 (s, 1H, H-22), 5.96 (t, J = 9.8 Hz, 1H, H-4′), 5.92 (bs, 1H, H-11), 5.84 (dd, J = 10.2 Hz, J = 3.4 Hz, 1H, H-3′), 5.80 (s, 1H, H-1), 5.68 (s, 1H, H-3), 5.59 (s, 1H, H-2′), 5.46 (s, 1H, H-1′), 5.41 (d, J = 12.3 Hz, 1H, H-19), 4.98 (d, J = 12.4 Hz, 1H, H-19), 4.87 (d, J = 17.5 Hz, 1H, H-21), 4.80 (d, J = 17.58 Hz, 1H, H-21), 4.45–4.42 (m, 1H, H-5′), 3.06 (bs, 1H), 2.59–1.52 (m, 16H), 2.18 (s, 1H, CH_3CO), 1.64 (s, 1H, CH_3CO), 1.47 (d, J = 6.14 Hz, 3H, H-6′), 1.13 (s, 3H, H-18). MS (FAB pos.) $(M + H + Na)^+$ m/z 1461.

3β,19-Bis-2-naphthoyloxy-1β,11α-bisacetoxy-14β-{(2,3, 4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5β-hydroxy-5β-card-20(22)-enolide (14-Rha/3,19-PN) (31b). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.65 (s, 1H), 8.54 (s, 1H), 8.52 (s, 1H), 8.42 (s, 1H), 8.10–7.33 (m, 30H), 6.35 (s, 1H, H-1), 5.98 (t, J = 10.0 Hz, 1H, H-4′), 5.94 (bs, 1H, H-22), 5.89 (dd, J = 10.3 Hz, J = 3.1 Hz, 1H, H-3′), 5.54 (s, 1H, H-3), 5.68 (s, 1H, H-2′), 5.32 (s, 1H, H-1′), 5.30–5.27 (m, 2H, H-19, H-11), 5.17 (d, J = 10.1 Hz, 1H, H-19), 4.90 (s, 2H, H-21), 4.46–4.36 (m, 1H, H-5′), 4.28 (s, 1H), 3.47–3.45 (bs, 1H), 3.36–3.32 (app t, 1H), 2.61–1.48 (m, 16H), 2.12 (s, 1H, CH_3CO), 1.73 (s, 1H, CH_3CO), 1.44 (d, J = 6.2 Hz, 3H, H-6′), 1.33 (s, 3H, H-18). MS (FAB pos.) $(M + H + Na)^+$ m/z 1461.

Glycosylation of 1β,19-Bis-2-naphthoyloxy-3β,11α-bisacetoxy-5,14β-tetrahydroxy-5β-card-20(22)-enolide (12).

A mixture of 1β,19-Bis-2-naphthoyloxy-3β,11α-bisacetoxy-5,14β-tetrahydroxy-5β-card-20(22)-enolide **12** (8 mg, 9.64 μmol) and 4 Å molecular sieves in CH_2Cl_2 (0.2 mL) was stirred for 10 min at room temperature and then cooled to −10°C. A solution of 2,3,4-tris-O-2-naphthoyl-β-L-rhamnopyranose 1-O-trichloroacetimidate **15** (8 mg, 10.38 μmol) in CH_2Cl_2 (0.2 mL) was added and the mixture was stirred for an additional 10 min. TMS.OTf (0.003 μL, 16.58 nmol) diluted in CH_2Cl_2 (22 μL) was then added and the reaction was almost instantaneous. The mixture was stirred for 15 min at −10°C and then warmed to 0°C over a 20-min period. It was then quenched with H_2O, extracted with CH_2Cl_2, and dried over Na_2SO_4. Plate (500 μm, 20 cm × 20 cm) chromatography (silica gel, 19:1 $CHCl_3$/MeOH) yielded rhamnosides **26b** (4.8 mg) and **32b** (2.8 mg).

1β,19-Bis-2-naphthoyloxy-3β,11α-bisacetoxy-5β-{(2,3,4 -tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-14β-hydroxy-5β-card-20(22)-enolide (5-Rha/1,19-PN) (26b). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.63 (s, 1H), 8.54 (s, 1H), 8.41 (s, 1H), 8.36 (s, 1H), 8.30–7.31 (m, 30H), 6.02 (s, 1H, H-1), 6.00 (s, 1H, H-22), 5.96 (t, J = 10.0 Hz, 1H, H-4′), 5.90 (bs, 1H, H-11), 5.85 (dd, J = 10.2 Hz, J = 3.0 Hz, 1H, H-3′), 5.59 (s, 1H, H-2′), 5.49 (d, J = 12.4 Hz, 1H, H-19), 5.46 (s, 1H, H-1′), 5.43 (s, 1H, H-3), 4.97 (d, J = 12.1 Hz, 1H, H-19), 4.87 (d, J = 17.2 Hz, 1H, H-21), 4.80 (d, J = 17.4 Hz, 1H, H-21), 4.47–4.42 (m, 1H, H-5′), 4.22 (s, 1H), 3.05–3.00 (m, 1H) 2.60–1.65 (m, 16H), 2.25 (s, 1H, CH_3CO), 1.60 (s, 1H, CH_3CO), 1.48 (d, J = 6.1 Hz, 3H, H-6′), 1.13 (s, 3H, H-18), MS (FAB pos.) $(M + 2H + Na)^+$ m/z 1462.

1β,19-Bis-2-naphthoyloxy-3β,11α-bisacetoxy-14β-{(2,3, 4-tris-O-2-naphthoyl-α-L-rhamnopyranosyl)oxy}-5β-hydroxy-5β-card-20(22)-enolide (14-Rha/1,19-PN) (32b). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.66 (s, 1H), 8.46 (s, 1H), 8.42 (s, 1H), 8.41 (s, 1H), 8.11–7.38 (m, 30H), 6.55 (s, 1H, H-1), 5.99 (t, J = 10.0 Hz, 1H, H-4′), 5.91 (s, 1H, H-22) 5.89 (dd, J = 10.4 Hz, J = 3.1 Hz, 1H, H-3′), 5.55 (s, 1H, H-2′), 5.45 (s, 1H, H-3), 5.34 (s, 1H, H-1′), 5.31 (s, 2H, H-19), 5.28–5.23 (m, 1H, H-11) 4.92 (s, 2H, H-21), 4.48–4.42 (m, 1H, H-5′), 4.40 (s, 1H), 3.37–3.33 (app t, 1H), 2.61–1.52 (m, 16H), 2.19 (s, 1H, CH_3CO), 1.61 (s, 1H, CH_3CO), 1.46 (d, J = 6.2 Hz, 3H, H-6′), 1.39 (s, 3H, H-18). MS (FAB pos.) $(M + 2H + Na)^+$ m/z 1462.

ACKNOWLEDGMENTS

We thank Professor W. Clark Still and Dr. Quentin McDonald, Columbia University, for helpful discussions on Molecular Modeling conformational analysis, and Professor N. Harada, Tohoku University, for providing the computation program for the CD calculations as well for stimulating discussions on theoretical analysis of CD. This work was supported by NIH HL52282 (G.T.H. and K.N.), NIH GM34509 (K.N.), and Bion Inc.

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