Synthesis and ^{13}C NMR chemical shift assignments of 2,2'-bipyridine-4,4'-dicarboxylates of bile acid methyl esters

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(received 15 Dec 99; accepted 13 Feb 00; published on the web 21 Feb 00)
DOI: http://dx.doi.org/10.3998/ark.5550190.0001.112

Abstract

Novel 2,2'-bipyridine-4,4'-dicarboxylates **3a**-**3d** of four bile acid methyl esters have been synthesized from 2,2'-bipyridine-4,4'-dicarboxylic acid **1** and the corresponding bile acid methyl esters (methyl lithocholate **2a**, methyl chenodeoxycholate **2b**, methyl deoxycholate **2c**, and methyl cholate **2d**). In addition to the desired products, 3α -(2,6-dichlorophenylcarboxy) bile acid methyl esters **4a**-**4d** were obtained. The structures of **3a**-**4d** have been ascertained by 1 D ^{1}H and ^{13}C NMR, 2 D PFG ^{1}H,^{13}C HMQC, and MALDI TOF MS. Molecular weights and ^{13}C NMR chemical shifts of **3a**-**4d** have been presented. The geometry of **3a** has been optimized semi-empirically at the PM3 level and it has been observed that the minimum energy structure of **3a** is a open type conformation due to lack of attractive intramolecular electrostatic interactions between the heads of the molecule which would have favoured the formation of cleft type structure. The synthesized bipyridine-bile acid conjugates are interesting structures from the molecular recognition point of view because they could have potential to form complexes with some transition metal ions, for example with silver, cadmium, and ruthenium.

Keywords: Bipyridine-bile acid, methyl lithocholate, methyl chenodeoxycholate

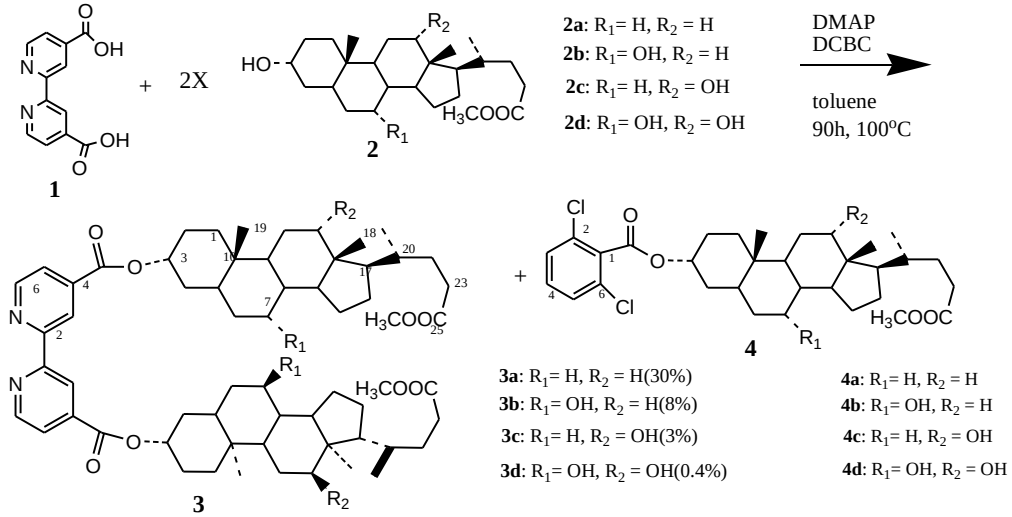
Introduction

Bile acids have shown to be versatile building blocks in tailoring supramolecular hosts.[1-7] They also have pharmacological potential to act as carriers of liver-specific drugs, absorption enhancers, and as cholesterol-lowering agents,[8] which have made bile acids extensively studied compounds in chemistry and medicine. We have recently reported synthetic procedures for preparing a lithocholaphane[9] and steroidal molecular clefts containing three arylcarboxy,[10] isomeric pyridine-*n*-carboxy,[11] and isomeric *n*-acetoxyphenylcarboxy (acetylsalicylate and its isomers)[12] moieties and their complexation tendencies towards silver(I)-cation.[11]

By introducing various heteroatoms, and especially nitrogen containing moieties, into the bile acid derivatives, their coordination spheres for cation binding can be greatly enhanced. From this point of view, 2,2'-bipyridine and its derivatives are very tempting structures. In this work we wish to report a synthetic route for 2,2'-bipyridine-4,4'-dicarboxylates of bile acid methyl esters using four common bile acids as starting materials: lithocholic acid (3α -hydroxy-5β -cholan-24-oic acid), chenodeoxycholic acid (3α ,7α -dihydroxy-5β -cholan-24-oic acid), deoxycholic acid (3α ,12α -dihydroxy-5β -cholan-24-oic acid), and cholic acid (3α ,7α ,12α -trihydroxy-5β -cholan-24-oic acid). An ongoing project is the preparation of tris(2,2'-bipyridine-4,4'-dicarboxylate)-ruthenium(II) chelates of these bile acid diesters. It is expected that bulky steroidal units will protect the ruthenium-bipyridine core from dioxygen quenching and prolong their photophysical excited lifetime, as in case of branched dendrimer Ru(II) complexes.[13]

Results and Discussion

The synthetic route to **3a-4d** is described in the Scheme. The syntheses of **3a-3d** were carried out using theYamaguchi reaction[14] as in our previous publications.[9,10] The yields of the 2,2'-bipyridine-4,4'-dicarboxylates of the bile acid methyl esters **3a-3d** were low because of the competitive benzoylation of the 3α -OH. The predominant products, except in the case of lithocholate, were the corresponding 3α -(2,6-dichlorophenyl-carboxy) bile acid methyl esters **4b-4d**. Therefore a more suitable catalyst, which would not react with the 3α -OH like DCBC, should be found, and the yields of desired products should be improved.



Scheme 1

An energetically optimized (PM3) structure of **3a** is plotted in Figure 1. As can be seen, the most favoured conformation of **3a** is open. This differs from that of isomeric pyridine-*n*-carboxy[11] and isomeric *n*-acetoxyphenylcarboxy derivatives[12] of bile acids where the closed "cleft" form is predominant due to a stabilization by π -stacking of aryl rings. However, it is possible that the

inclusion of the guest molecule could induce the cleft type conformation as Kohmoto *et al*. observed to happen for their naphthalene-1,4,5,8-tetracarboxylic dianhydride bridged 3α -aminocholanoate derivative.[15]

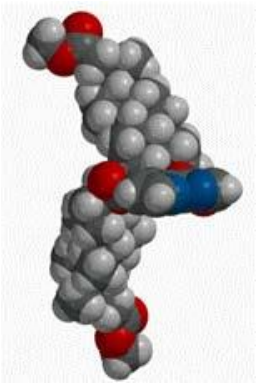


Figure 1. Van der Waals surface of **3a** optimized at the PM3 level.

Because MO-calculations of **3a** were very computational time consuming, the calculations of compounds (**3b-d**) were not performed, but it is reasonable to assume that the additional hydroxyl at the 7α - and 12α -positions would not markedly influence the conformation of the compounds. In the future the compounds **3a-3d** will be subjected to complexation studies with transition metal cations and some small molecules. The goals of these studies are to find novel catalysts, proper structures for molecular and ionic recognition and intermediates for larger host molecules suitable for supramolecular chemistry.

Experimental Section

General Procedures. The purity of compounds **3a-3d** was checked by thin layer chromatography using Merck silica gel 60 F_{254} plates (visualization with conc. H_2SO_4/MeOH, 1:1). Column chromatography was performed using Merck silica gel 60, particle size 0.040-0.063 mm, using acetone, CH_2Cl_2, and acetone:$CHCl_3$, and acetone:CH_2Cl_2 mixtures as eluent. The ^{1}H, ^{13}C and ^{13}C DEPT-135 NMR spectra were run with a Bruker Avance DPX 250 NMR spectrometer equipped with a 5 mm diameter broad band inverse probehead working at 250.13 MHz in ^{1}H and 62.90 MHz in ^{13}C experiments. The z-PFG ^{1}H,^{13}C HMQC experiments were recorded by a Bruker Avance DRX 500 NMR spectrometer equipped with an inverse detection 5 mm broad band probehead operating at 500.13 MHz in ^{1}H and 125.77 MHz in ^{13}C, respectively, in 0.05-0.1 M $CDCl_3$-solutions at 30 ^{o}C. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent: δ ^{1}H ($CHCl_3$) = 7.26 ppm and δ ^{13}C ($CDCl_3$) = 77.00 ppm. The ^{13}C NMR chemical shift assignments of **3a-4d** (Table 1) are based on ^{13}C DEPT 135 and z-PFG ^{1}H,^{13}C HMQC experiments. Molecular weights of **3a-4d** were determined by the MALDI-TOF technique with a Bruker Proflex equipment in the Department of Chemistry, University of Joensuu, Finland. The geometry of the **3a** was fully optimized at the semi-empirical PM3 level[16]

on a Silicon Graphics O2 workstation by using SPARTAN (Version 5.0)[17] and Gaussian 98[18] software.

Table 1. ^{13}C NMR chemical shifts (ppm from $CDCl_3$, δ = 77.00) of 2,2'-bipyridine-4,4'-dicarboxylates of bile acid methyl esters (**3a**-**3d**) and 3α -(2,6-dichlorophenylcarboxy) bile acid methyl esters (**4a**-**4d**)

Carbon[a]	**3a**	**3b**	**3c**	**3d**	**4a**	**4b**	**4c**	**4d**
1	35.06	35.07[f]	34.95	35.20	34.95	34.80[f]	34.74	34.70[g]
2	26.65[b]	26.60	27.46[g]	26.73	26.46[b]	26.44	27.27[g]	26.49
3	76.25	76.04	76.08	76.08	76.62	76.68	76.50	76.55
4	32.24	34.34	32.25	34.92[h]	31.90	34.33	31.77	34.62[h]
5	42.01	41.21	42.03	42.17[i]	41.95	41.24	41.86	41.77[i]
6	27.04[b]	34.87[f]	26.63[g]	34.39[h]	26.93[b]	34.73[f]	26.24[g]	34.42[h]
7	26.33[b]	68.24	26.06[g]	68.24	26.25[b]	67.91	25.88[g]	68.17
8	35.84[c]	39.28	36.09[c]	39.69	35.73[c]	39.22	35.85[c]	39.27
9	40.50	32.75	33.82	26.94	40.37	32.63	33.49	26.37
10	34.66	35.00	34.24	34.78	34.56	34.92	34.04	34.57
11	20.90	20.50	28.81	28.53	20.77	20.45	28.57	27.85
12	40.13	39.41	73.20	72.90	40.05	39.42	72.83	72.90
13	42.78	42.56	46.58	46.63	42.65	42.42	46.33	46.37
14	56.48[d]	50.30	48.35	41.35[i]	56.39[d]	50.17	48.03	41.21[i]
15	24.18	23.55	23.63	23.17	24.10	23.45	23.46	23.07
16	28.18	27.99	27.02[g]	27.44	28.08	27.96	26.80[g]	27.36
17	56.04[d]	55.69	47.47	47.31	55.91[d]	55.61	47.12	46.99
18	12.05	11.63	12.79	12.62	11.96	11.59	12.56	12.32
19	23.31	22.58	23.16	22.60	23.23	22.54	22.95	22.22
20	35.37[c]	35.22	35.10[c]	35.14	35.26[c]	35.15	34.92[c]	35.15
21	18.28	18.12	17.40	17.41	18.18	18.04	17.13	17.17
22	31.08[e]	30.88[e]	31.10[e]	31.09[e]	30.96[e]	30.79[e]	30.88[e]	30.88[e]
23	31.04[e]	30.88[e]	30.95[e]	30.94[e]	30.92[e]	30.79[e]	30.75[e]	30.76[e]
24	174.74	174.52	174.65	174.65	174.55	174.40	174.48	174.71
25	51.44	51.30	51.49	51.51	51.32	51.22	51.29	51.36
CO(aroyl)	164.67	164.56	164.75	164.80	164.01	163.97	164.02	164.09
Aryl carbons								
1	-	-	-	-	133.98	133.92	133.87	134.08
2	156.60	156.41	156.65	156.65	131.65	131.49	131.54	131.63
3	120.55	120.41	120.58	120.61	127.74	127.61	127.66	127.64
4	139.49	139.27	139.38	139.41	130.54	130.44	130.47	130.46
5	123.29	123.09	123.26	123.25	127.74	127.61	127.66	127.64

6	149.98	149.80	150.04	150.03	131.65	131.49	131.54	131.63

[a] Owing to the C_2 symmetry, δ (^{13}C-*n*) = δ (^{13}C-*n*') in **3a**-**3d**.

[b-i]Assignments may be interchanged.

2,2'-Bipyridine-4,4'-dicarboxylic acid[19] (1)

The commercially available (Aldrich, 99%) 4,4'-dimethyl-2,2'-bipyridine (2.50 g, 13.57 mmol) in 25% sulphuric acid (132 mL, distilled water) was cooled to 5 ºC and treated with one portion of $KMnO_4$ (5.00 g, 31.64 mmol) while being stirred. After a further 30 min of stirring at 5 ºC, cooling was discontinued and the temperature of the mixture slowly rose to 35 ºC and kept in this temperature for 20 min. Then the mixture was cooled again to 5 ºC and a second portion of $KMnO_4$ (5.00 g, 31.64 mmol) was added. After a further 10 min the mixture was refluxed for 12 h at 130 ºC. Then the excess $KMnO_4$ was reduced by potassium metabisulfite (0.05 g) and after cooling the grey precipitate was filtered and dried *in vacuo*; yield: 1.67 g (50%). The purity of the product was checked by ^{1}H and ^{13}C NMR spectroscopy in NaOD/D_2O. The impurities 4,4'-dimethyl-2,2'-bipyridine and 4'-methyl-2,2'-bipyridine-4-carboxylic acid were present in under 2%.

Methyl lithocholate (2a). To a solution of lithocholic acid (3α -hydroxy-5β -cholan-24-oic acid, 5.00 g, 13.28 mmol) in methanol (30 mL) was added four drops of conc. sulphuric acid and the mixture was refluxed for 24 h. After cooling, $CHCl_3$ (60 mL) was added and the $CHCl_3$ layer was extracted with sat. aq. $NaHCO_3$ (4 x 20 mL), washed with water (1 x 25 mL), dried ($MgSO_4$), and evaporated to dryness; yield: 5.16 g (96%). The other bile acid methyl esters **2b**-**2d** were synthesized by appropriate modification of this procedure; yields: **2b** (90%), **2c** (90%), and **2d** (96%). The purity of the esters was checked by ^{13}C NMR spectroscopy in $CDCl_3$.

2,2'-Bipyridine-4,4'-dicarboxylates of bile acid methyl esters (3a-d); general procedure. To a solution of **2a** (2.17 g, 5.39 mmol) and **1** (0.66 g, 2.70 mmol) in sodium dried toluene (150 mL) was added 4-(*N*,*N*-dimethyl)aminopyridine (DMAP, 2.50 g, 20.46 mmol) and the mixture was heated to 100 ºC. Then 2,6-dichlorobenzoyl chloride (DCBC, 1.20 g, 5.73 mmol) was added and the mixture was kept at 100 ºC for 90 h.[14] After the reaction period the solvent was evaporated *in vacuo*. The crude product was dissolved in CH_2Cl_2 (80 mL) and extracted with sat. aq. $NaHCO_3$ (2 x 60 mL), washed with water (1 x 60 mL), dried ($MgSO_4$), and evaporated to dryness. Compounds **3b**-**3d** were synthesized by adaptation of this procedure.

Dimethyl-3α ,3'α -bis(2,2'-bipyridine-4,4'-dicarboxy)-5β ,5'β -dicholan-24,24'-dioate (3a). Purification. 3a was purified by sequential column chromatography: i) silica gel, acetone/$CHCl_3$ (5:95), ii) silica gel, CH_2Cl_2, and iii) silica gel, acetone; yield: 0.81 g (30.3%). MS (MALDI-TOF): m/z = 989.17 $[M+H]^+$. Methyl 3α -(2,6-dichlorophenylcarboxy) lithocholate (**4a**) was also obtained (0.40 g); MS (MALDI-TOF): m/z = 585.50 $[M+Na]^+$.

Dimethyl-3α ,3'α -bis(2,2'-bipyridine-4,4'-dicarboxy)-7α ,7'α -dihydroxy-5β ,5'β -dicholan-24,24'-dioate (3b). Purification. 3b was purified by sequential column chromatography: i) silica gel, acetone/CH_2Cl_2 (4:96); pure **3b** was obtained 0.1 g, and ii) silica gel, acetone/$CHCl_3$ (5:95); pure **3b** was obtained 0.1 g. Total yield: 0.20 g (8.0%). MS (MALDI-TOF): m/z = 1022.22 $[M+H]^+$. Methyl 3α -(2,6-dichlorophenylcarboxy) chenodeoxycholate (**4b**) was also obtained

(1.00 g); MS (MALDI-TOF): m/z = 601.74 $[M+Na]^+$.

Dimethyl-3α, 3'α -bis(2,2'-bipyridine-4,4'-dicarboxy)-12α ,12'α -dihydroxy-5β ,5'β -dicholan-24,24'-dioate (3c). Purification. 3c was purified by sequential column chromatography: i) silica gel, acetone/CH_2Cl_2 (4:96), and ii) silica gel, acetone/$CHCl_3$ (4:96); yield: 0.07 g (2.9%). MS (MALDI-TOF): m/z = 1020.91 $[M+H]^+$. Methyl 3α -(2,6-dichlorophenylcarboxy) deoxycholate (**4c**) was also obtained (1.42 g); MS (MALDI-TOF): m/z = 601.53 $[M+Na]^+$.

Dimethyl-3α ,3'α -bis(2,2'-bipyridine-4,4'-dicarboxy)-7α ,7'α ,12α ,12'α -tetrahydro-xy-5β, 5'β -dicholan-24,24'-dioate (3d). Purification. 3d was purified by sequential column chromatography: i) silica gel, acetone/$CHCl_3$ (5:95), and ii) silica gel, acetone/$CHCl_3$ (5:95); yield: 0.01 g (0.4%). MS (MALDI-TOF): m/z = 1054.15 $[M+H]^+$. Methyl 3α -(2,6-dichlorophenylcarboxy) cholate (**4d**) was also obtained (1.40 g); MS (MALDI-TOF): m/z = 617.44 $[M+Na]^+$.

Acknowledgements

We are grateful to Prof. P. Vainiotalo and Spec. Lab. Tech. Ritva Romppanen (Univ. Joensuu, Finland) for running the MALDI-TOF mass spectra and to Mr. R. Kauppinen for help in running NMR spectra. The Academy of Finland has financially supported this work, which also is gratefully acknowledged.

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