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Sulfate esters of morphine derivatives: Synthesis and characterization

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ABSTRACT

Sixteen 3-*O*- and 6-*O*-sulfate esters of morphine, codeine and some of their *N*-methyl quaternary derivatives were synthesized by means of sulfation with pyridine–SO_3 complex and sulfuric acid/*N*,*N*′-dicyclohexylcarbodiimide. Complete ^{1}H- and ^{13}C-NMR assignments are given for each of the synthesized compounds based on one- and two-dimensional homo- and heteronuclear measurements. Comparative analysis of chiral properties by circular dichroism and optical rotatory dispersion revealed characteristic differences in the spectra due to changes in charge, polarity and intramolecular association by strong hydrogen bonds in aqueous solution. The synthesized sulfate esters are prospective peripheral analgesics lacking central side effects and are also useful as reference substances for various analytical studies involving sulfate ester metabolites.

1. Introduction

Central and peripheral opioid receptors are primary targets for pain management. Therapeutic potential and demand of opioid analgesics have initiated tremendous scientific efforts, which have resulted in the development of a number of new opioid analgesics and an expansion of knowledge on the opioid pharmacology (Eguchi, 2004). The structural features of morphine skeleton have long been the basis of successful drug developments. There is, however, a great need for morphine-like molecules with improved efficacy, *in vivo* half lives, bioavailability and reduced side-effects such as tolerance liability, depressed respiration, and reduced gastrointestinal motility. The prospect for highly polar or charged opioid analogues with limited brain penetration to produce a peripherally mediated analgesic effect in inflammatory conditions has long been a pharmacologic desire and has recently been studied in several laboratories (Guan et al., 2008; Smith, 2008; Hernández et al., 2009; Obara et al., 2009; Zheng, 2010). Such highly polar, zwitterionic molecules are found among the naturally occurring metabolites (i.e. glucuronides and sulfates) of morphine and its derivatives (Milne et al., 1996; Wittwer and Kern, 2006; Coller et al., 2009). Morphine-3-*O*-sulfate and naloxone-3-*O*-sulfate have been isolated from biological samples (Ober and Fujimoto, 1972; Yeh et al., 1977). It is therefore important to characterize known metabolites and to synthesize new analogous sulfate esters to better understand their physicochemical properties. Despite the apparent inability of sulfate metabolites to cross the blood–brain barrier due to the presence of the charged sulfate ester group, several studies have indicated that some sulfate esters of morphine derivatives are of pharmacological interest (Mori et al., 1972; Brown et al., 1985; Preechagoon et al., 1998a,b; Zuckerman et al., 1999). Introduction of a sulfate ester group at the C-6 hydroxyl has been shown to significantly increase the analgesic potency of morphine (Brown et al., 1985). The present paper focuses on sulfate esters that may be effective as peripherally acting opiate analgesics.

For the synthesis of sulfate esters a number of approaches have been reviewed (Al-Horani and Desai, 2010). The most common methods are the reaction of the phenol or the alcohol with chlorosulfonic acid in pyridine and with trimethylamine–SO_3 complex or pyridine–SO_3 complex in DMF, 1,4-dioxane or pyridine (Kaspersen and Van Boeckel, 1987). Successful syntheses of sulfate esters using pyridine–SO_3 complex are reported for morphine derivatives, carbohydrates and steroids (McKenna and Norymberski, 1957; Popek and Lis, 2002; Crooks et al., 2006). While some of the compounds are known, this paper provides detailed NMR and CD spectroscopic analysis of a large set of sulfate esters which could serve as basis for further physicochemical studies (Visky et al., 2000; Mazák et al., 2009) and as a tool for the identification of sulfate metabolites in various biological samples.

2. Results and discussion

The synthesis of sixteen sulfate esters of morphine and codeine derivatives was accomplished. The reaction involves sulfation

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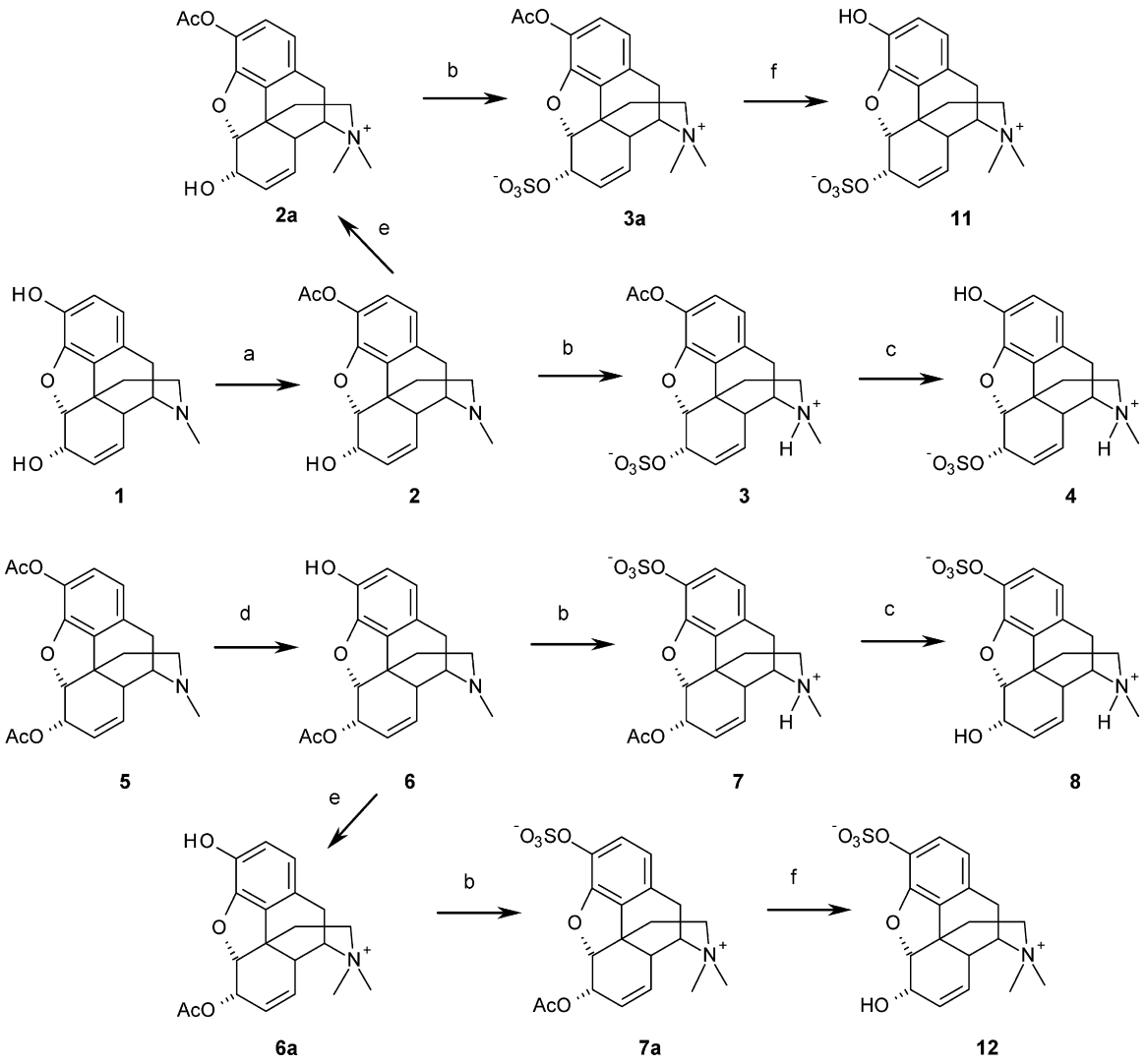


Scheme 1. Synthesis of morphine monosulfate esters (**4**,**8**,**11**,**12**). (a) Acetic anhydride, $NaHCO_3$, H_2O, r.t., 1 h. (b) Pyridine-SO_3, pyridine, 60 °C, 3.5 h. (c) 10% aq. NaOH, MeOH, r.t., 1 h. (d) Hydroxylamine hydrochloride, EtOH, 60 °C, 45 min. (e) MeI, acetone, 40 °C, 4 h. (f) 20% aq. K_2CO_3 solution, r.t., 1 h.

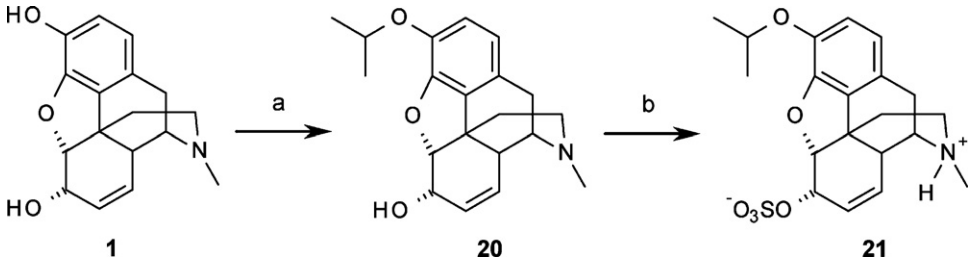
of the respective C-6 or C-3 hydroxyl functions, upon stirring the appropriate morphine derivative with 3 equivalents of pyridine–SO_3 complex in dry pyridine at 60 °C for 3.5 h.

Morphine and dihydromorphine have two hydroxyl groups (C-3 phenolic and C-6) that can be readily sulfated. The reactivity difference of the two hydroxyl groups is not enough, however, for direct regioselective sulfation to produce the monoesters. In order to obtain the sulfate esters (**4, 8**–**10**), we used acetyl protecting groups at the C-3 phenolic or C-6 hydroxyl groups. Selective acetylation of the phenolic hydroxyl moiety was achieved upon stirring morphine (**1**) with acetic anhydride in aqueous medium in the presence of excess sodium hydrogen carbonate (Welsh, 1954). For the synthesis of morphine-3-*O*-sulfate (**8**) protection of the C-6 hydroxyl group was achieved by selective hydrolysis of 3,6-*O*-diacetylmorphine (**5**) with hydroxylamine hydrochloride in ethanol (Wright, 1941). The sulfation of the appropriately acetylated substances was achieved by the general method using pyridine–SO_3 with subsequent alkaline hydrolysis of the protecting group (Scheme 1).

The same pathway was followed for the synthesis of the respective dihydromorphine analogues (**9, 10**). During the synthesis of the quaternary 6-*O*- and 3-*O*-sulfate esters of morphine (**11, 12**) and dihydromorphine (**13, 14**) introduction of the appropriate acetyl protecting group was followed by methylation of the *N*-atom upon heating the morphine derivatives with small excess methyl iodide in acetone. Due to the limited solubility of the quaternary compounds in pyridine, sulfation was carried out in pyridine/DMF 2:1 mixture at 100 °C. Deacetylation of the quaternary morphine and dihydromorphine sulfate esters was achieved by 20% aqueous potassium carbonate solution.

A set of sulfate esters of morphine 3-*O*-ethers has been synthesized. A new and potentially pharmacologically active morphine derivative, 3-*O*-isopropylmorphine (**20**) was prepared by heating morphine (**1**) with 2-iodopropane in the presence of sodium ethoxide in dry ethanol (Scheme 2). Isocodeine, the 6β epimer of codeine was prepared by Mitsunobu reaction (Simon et al., 1991). Direct sulfation using the general method described above yielded the desired sulfate esters of codeine (**15**), dihydrocodeine (**16**), their quaternary derivatives (**17, 18**), ethylmorphine (**19**), 3-*O*-isopropylmorphine (**21**) and isocodeine (**22**).

The reaction of morphine (**1**) with 6 equivalents of pyridine–SO_3 complex, along with the esterification of both morphine-3-*O*-sulfate (**8**) and morphine-6-*O*-sulfate (**4**) by pyridine–SO_3 have afforded mixtures of the diester morphine-3,6-*O*-disulfate (**23**) and the monoester, respectively. Since most physicochemical properties of these highly polar, zwitterionic substances are very similar and are therefore inseparable by common tech-



Scheme 2. Synthesis of 3-*O*-isopropylmorphine-6-*O*-sulfate (**21**). (a) 2-iodopropane, NaOEt, EtOH, reflux, 4 h. (b) Pyridine-SO_3, pyridine, 60 °C, 3.5 h.

niques (i.e. recrystallization, chromatography) we synthesized the new diester morphine-3,6-*O*-disulfate by direct sulfation of morphine (**1**) by concentrated sulfuric acid in DMF using *N,N′*-dicyclohexylcarbodiimide as the coupling agent (Linder and Fishman, 1973) (Scheme 3).

2.1. Spectral analyses

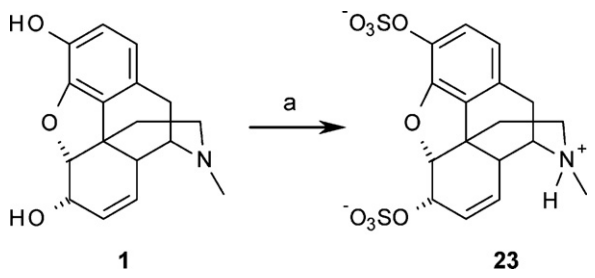
The structural properties of the synthesized compounds were studied by MS, NMR, UV, CD and ORD spectroscopy. All NMR signals in the ^{1}H and ^{13}C spectra of the synthesized derivatives were completely assigned based on one- and two-dimensional homo- and heteronuclear NMR experiments (COSY, TOCSY, HSQC, HMBC and NOESY) (Tables 1 and 2). The effect of the sulfate ester groups on the chemical shifts of several atoms due to changes in charge distribution, steric interactions and ring conformation was studied. For comparison, assignment of some of the unesterified substances was also done based on spectra recorded in DMSO-d_6.

2.2. NMR spectroscopy

In the ^{1}H-NMR spectra of sulfate esters, the aromatic H-1 and H-2 appear as doublets due to spin-spin 3-bond coupling. Significant downfield shifts were observed in 3-*O*-esters (**8, 10, 12, 14**) for H-1 (about +0.20 ppm) and for H-2 (from +0.53 ppm to +0.70 ppm). This is attributed to steric crowding and decreased electron density of the aromatic ring, due to the electron-withdrawing effect of the sulfate group (Brown et al., 1985).

Significant downfield shifts in 6-*O*-esters were observed for H-7, H-8, H-14, H-5 and H-6, the latter being most significant for most of the studied 6-*O*-esters. In isocodeine-6-*O*-sulfate (**22**) the sulfate ester moiety is axial, causing increased steric crowding and larger chemical shifts for H-5, H-7 and H-8 relative to those of codeine-6-sulfate (**15**). The signal for H-6 was detected as a broad, irresolvable multiplet in the spectra of the described sulfate esters; this is due to the electromagnetic effect of the charged sulfate ester moiety. For H-9 and H-16 remarkably high downfield shifts were observed in all of the studied substances (from +0.50 ppm to +0.90 ppm).

The signals for *N*-methyl hydrogens were shifted downfield by approximately +0.60 ppm compared to the unesterified derivatives. The *N*-methyl shifts for the zwitterionic sulfate esters are significantly higher than those of the respective salts in which the *N*-atom is also protonated. In quaternary sulfate esters the shifts for both the axial and the equatorial *N*-methyl (Iorio et al., 1984) groups were higher than those of the respective unesterified substances, nevertheless, the difference was less significant.



Scheme 3. Synthesis of morphine-3,6-*O*-disulfate ester (**23**). (a) *N,N′*-dicyclohexylcarbodiimide, *cc.* sulfuric acid, DMF, 0 °C, 15 min.

In ^{13}C-NMR spectra, the sulfation of the phenolic hydroxyl group causes downfield shifts for C-2 and C-4 (from +3.34 ppm to +7.20 ppm). The signals for C-3 were shifted upfield by approximately 3.20 ppm. In contrast, esterification of the C-6 hydroxyl group results in downfield shifts for C-6 and slight upfield shifts for C-7 and C-8. Slight upfield shifts could also be observed for C-14 and the carbon of the N^+-methyl group in all of the studied substances.

A second set of signals for H-6–H-9 and *N*-Me with an integral ratio of approximately 1:4 was observed in the 1H spectra of 7-8 unsaturated 6-*O*-sulfates containing tertiary *N*-atom (**4, 15, 19, 21, 22**). The respective peaks merged upon heating the sample suggesting a slow and hindered conformational equilibrium of the rings C and D between the boat/chair and skew-boat/skew-chair conformations as previously observed in solid state ^{13}C-NMR investigations (Kottayil, 1993). Intensive cross-peaks in the NOESY spectrum of (**4**) between the hydrogen atoms of the phenolic hydroxyl, the protonated nitrogen atom and water may suggest the presence of a water molecule bound to both groups and also to the sulfate ester by strong hydrogen bonds.

2.3. Chiroptical spectroscopy

Concerning structural elements of chiral properties, the circular dichroism (CD) and optical rotatory dispersion (ORD) methods have proven to be sensitive tools in the analysis of morphine derivatives (Bowen, 1980; Crone and Purdie, 1981; Han and Purdie, 1986; Purdie et al., 1989; Gergely et al., 2004). Morphine sulfate esters contain one substituted benzene ring as UV chromophore, two hydroxyls (phenolic and cycloaliphatic) capable of forming hydrogen bond interactions and an ether group. The highly polar, permanently charged zwitterionic character could affect chiral properties by changing solvatation, forming hydrogen bonds or salt bridges between the charged functional groups.

Fig. 1 shows the CD, ORD and UV spectra of selected 3-*O*-sulfate and 6-*O*-sulfate derivatives. CD and ORD spectra show remarkable differences that can be considered as consequences of the geometric factors responsible for intensifying Cotton effects of π–π^* and n–π^* transitions originating from the different substituents and conformational states of the morphine C and D rings. The UV spectra of the 6-*O*-sulfates (**4, 11, 15, 22**) are identical to morphine (**1**) (λ_{max} = 285.2 nm), but hypsochromic shifts are detected in the spectrum of morphine-3-*O*-sulfate (**8**) (λ_{max} = 281.8 nm).

Upon comparing the CD spectral properties of morphine with morphine *O*-sulfates, similarities are found in the spectra of 6-*O*-sulfate derivatives (**1, 4, 11, 15, 22, 23**) but characteristic differences are observed in the spectra of 3-*O*-sulfate derivatives (**8, 12**). The studied compounds show low negative Cotton effect at the ^1L_b

Table 1
^{1}H-NMR assignments of the synthesized compounds.

Compound	^{1}H-NMR															
	H1[a]	H2[a]	H5[b]	H6[c]	H7[d]	H8[e]	H9[f]	H10[g]		H14[h]	H15[i]		H16[j]		NMe	OR
4	6.46	6.56	4.97	4.59	5.79	5.31	4.16	2.75	3.21	2.83	1.96	2.21	2.86	3.32	2.91	
8	6.56	6.97	4.87	4.11	5.62	5.22	4.11	2.76	3.25	2.82	1.95	2.19	2.84	3.30	2.91	
9	6.55	6.66	4.78	4.23	1.00eq 1.55ax	0.82ax 1.64eq	3.84	2.82	3.17	2.42	1.81	2.02	2.74	3.20	2.87	
10	6.64	6.96	4.62	3.94	1.36eq 1.53ax	1.09ax 1.25eq	3.79	2.81	3.16	1.91	1.66	2.00	2.59	3.15	2.82	
11	6.43	6.56	4.95	4.83	5.76	5.29	4.10	2.81	3.46	3.47	1.84	2.58	3.14	3.36	3.27eq 3.41ax	
12	6.55	6.97	4.84	4.15	5.60	5.22	4.10	2.82	3.51	3.41	1.86	2.55	3.13	3.40	3.28eq 3.38ax	
13	6.52	6.66	4.77	4.45	0.96eq 1.59ax	0.89ax 1.59eq	3.78	2.92	3.40	2.28	1.68	2.44	3.01	3.24	3.22eq 3.32ax	
14	6.65	6.98	4.60	4.20	1.45eq 1.54ax	1.09ax 1.24eq	3.81	2.89	3.43	2.85	1.60	2.32	2.89	3.28	3.20eq 3.32ax	
15	6.59	6.75	5.00	4.62	5.76	5.29	4.17	2.78	3.26	2.87	1.96	2.24	2.86	3.31	2.93	3.73[k]
16	6.67	6.84	4.80	4.28	1.12eq 1.51ax	0.83ax 1.59eq	3.86	2.85	3.18	2.42	1.80	2.03	2.71	3.20	2.86	3.79[k]
17	6.56	6.74	5.00	4.84	5.74	5.29	4.11	2.84	3.49	3.49	1.86	2.60	3.13	3.37	3.28eq 3.40ax	3.73[k]
18	6.65	6.85	4.79	4.47	1.07eq 1.56ax	0.84ax 1.55eq	3.81	2.95	3.44	3.04	1.69	2.42	2.98	3.27	3.22eq 3.33ax	3.80[k]
19	6.56	6.73	4.98	4.60	5.79	5.32	4.17	2.79	3.26	2.85	1.98	2.23	2.85	3.32	2.91	4.01[l] 1.29[m]
20	6.54	6.66	4.87	4.17	5.69	5.29	3.35	2.30	3.04	2.66	1.87	2.06	2.40	2.59	2.44	4.52[n] 1.29[o] 1.34[o]
21	6.56	6.70	4.98	4.59	5.79	5.32	4.17	2.79	3.25	2.85	1.97	2.22	2.86	3.31	2.91	4.57[n] 1.21[o]
22	6.64	6.79	5.10	4.49	5.94	5.65	4.16	2.78	3.26	3.11	1.94	2.15	2.78	3.31	2.91	3.75[k]
23	6.53	7.14	4.97	4.61	5.75	5.30	4.15	2.84	3.26	2.95	1.91	2.25	2.89	3.29	2.88	
Codeine	6.46	6.61	4.68	4.11	5.54	5.25	4.11	2.23	2.92	2.55	1.62	1.98	2.22	2.45	2.30	3.72[k]
Dihydrocodeine	6.53	6.68	4.46	3.81	1.17eq 1.30ax	0.90ax 1.38eq	2.93	2.27	2.83	2.10	1.44	1.79	2.06	2.35	2.25	3.76[k]
Dihydromorphine	6.41	6.52	4.44	3.80	1.16eq 1.30ax	0.89ax 1.39eq	2.92	2.24	2.82	2.10	1.44	1.78	2.08	2.35	2.25	
Morphine	6.34	6.44	4.66	4.09	5.53	5.24	3.24	2.20	2.88	2.53	1.62	1.97	2.23	2.44	2.29	

[a] *d*, $J_{1,2} \sim 8.0$ Hz, 1 H.
[b] *d*, $J_{56} \sim 6.0$ Hz, 1 H.
[c] *br d*, $J_{6,7} \sim 2.0$ Hz, 1 H.
[d] 7–8 unsaturated: *d*, $J_{7,8} \sim 9.5$ Hz, 1 H; 7–8 saturated: *dd*, $J_{7eq,7ax} \sim 14.5$ Hz, $J_{7eq,8eq} \sim 3.0$ Hz, 2 H.
[e] 7–8 unsaturated: *d*, $J_{7,8} \sim 9.5$ Hz, 1 H; 7–8 saturated: *dd*, $J_{8eq,8ax} \sim 14.5$ Hz, $J_{7eq,8eq} \sim 3.0$ Hz, 2 H.
[f] *d*, $J_{8,9} \sim 4.5$ Hz, 1 H.
[g] *dd*, $J_{10a,10b} \sim 20.0$ Hz, $J_{9,10} \sim 5.5$ Hz, 2 H.
[h] *s*, 1 H.
[i] *dd*, $J_{15a,15b} \sim 13.0$ Hz, $J_{1516} \sim 3.5$ Hz, 2 H.
[j] *dd*, $J_{16a,16b} \sim 13.0$ Hz, $J_{15,16} \sim 3.5$ Hz, 2 H.
[k] *s*, 3 H, OCH_3.
[l] *m*, 1 H, OCH_2CH_3.
[m] *t*, $J = 7.0$ Hz, 3 H, OCH_2CH_3.
[n] *m*, 1 H, $OCH(CH_3)_2$.
[o] *d*, $J = 5.0$ Hz, 6 H, $OCH(CH_3)_2$.

absorption band around 286 nm (−75 to −13 deg cm^2 dmol^{-1}), medium intensive positive molar ellipticity at the ^1L_a absorption band between 242 and 246 nm (+250 to +450 deg cm^2 dmol^{-1}) for 6-*O*-sulfates, between 235 and 236 nm for the 3-*O*-sulfates (+120 to +160 deg cm^2 dmol^{-1}) and very intensive ^1B absorption band between 210 and 218 nm (−540 to −1300 deg cm^2 dmol^{-1}) in the CD spectra. The most remarkable differences are found in the high intensity ^1B absorption bands. The absolute values of these negative ellipticities increase in the order **1** < **8** < **12** < **23** < **4** < **22** < **15**. 3-*O*-sulfation caused moderate hypsochromic shift as compared to morphine in the same absorption band. The peak wavelengths detected for the ^1B band are in the following order: **8** ≈ **12** (211.0 nm) < **22** (213.4 nm) < **23** (215.5 nm) < **1** ≈ **4** (217.0 nm) < **15** (217.4 nm). While λ^0 (wavelength with zero ellipticity) occurred at the same wavelengths for (**1, 4, 11, 15, 22, 23**) (231 nm and 263 nm), λ^0 values for (**8, 12**) are shifted towards shorter wavelengths (220 nm) between the ^1B and ^1L_a absorption bands, and longer wavelengths (276 nm) between ^1L_a and ^1L_b bands. These characteristic spectral features make 3-*O*-sulfates distinguishable from 6-*O*-sulfates.

The ORD spectra of morphine and selected morphine *O*-sulfate derivatives were compared. Morphine sulfates exhibit broad maxima around 245–260 nm and intensive minima around 280–300 nm. The only remarkable difference is found in the spectrum of (**22**), the 6β epimer of (**15**). On the basis of the ORD spectral similarities the absolute stereochemistry of asymmetric centers in morphine sulfates are identical to those of their parent molecules and have not changed under the conditions of the different sulfation reactions.

Remarkable changes in intensities and the shape of CD and ORD curves suggest that interactions between the C-3 and C-6 groups may be attributed to modification or stabilization of the C-ring conformations and hindered rotation of the sulfate ester group around the O-S bond. The more polar sulfate group in morphine derivatives causes expansion in solvate shell, which could fold back to the 3-hydroxyl group of morphine derivatives by insertion of water molecule or ammonium complex formation. This type of association was supported by NOE experiments, where correlation was found between the C-3 hydroxyl, C-6 sulfate and water. Order of hypsochromic shifts in the CD spectra could also provide expla-

Table 2
^{13}C-NMR assignments of the synthesized compounds.

Compound	^{13}C-NMR																	
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	NMe	OR
4	119.10	117.22	139.18	146.39	89.13	70.56	132.59	125.42	59.63	20.88	121.80	128.71	41.70	38.28	32.71	46.20	40.55	
8	118.93	123.29	134.94	150.24	89.97	65.13	135.83	124.29	59.64	20.90	127.36	129.47	40.80	37.73	32.25	46.20	40.33	
9	118.95	117.82	138.83	146.04	87.62	71.11	20.30	19.65	60.31	20.30	122.16	127.63	41.36	33.62	34.64	46.02	40.49	
10	118.47	124.54	133.72	150.26	88.99	65.01	26.84	16.82	60.24	20.35	123.45	129.18	41.59	30.91	33.50	46.70	40.04	
11	118.95	117.26	139.39	146.18	89.54	70.26	132.62	125.46	68.75	23.31	121.12	128.84	41.53	33.04	29.18	54.98	49.54ax 53.60eq	
12	118.91	123.47	135.01	150.04	90.36	65.09	135.52	124.60	68.78	23.55	126.72	129.47	40.73	32.75	28.95	55.11	49.65ax 53.56eq	
13	118.82	117.93	139.04	146.03	87.63	70.39	20.17	19.58	69.27	22.21	124.60	127.99	41.22	27.60	30.51	54.12	49.26ax 53.15eq	
14	118.48	124.49	134.19	150.25	89.07	64.86	27.13	17.83	69.95	23.30	124.33	128.18	41.08	32.83	30.33	56.34	49.95ax 53.60eq	
15	119.26	113.99	141.92	147.31	89.51	70.43	132.59	125.52	59.54	20.88	123.85	128.94	41.58	38.18	32.62	46.39	40.61	55.97[a]
16	119.12	115.53	141.53	147.14	87.75	70.98	21.21	19.27	60.33	20.30	124.18	127.91	41.20	34.20	34.15	46.20	40.50	56.57[a]
17	119.09	114.05	142.00	147.13	89.92	70.10	132.67	125.46	68.69	23.31	129.06	132.67	41.39	32.96	29.06	54.92	49.58ax 53.61eq	55.99[a]
18	118.95	115.60	141.56	146.98	88.16	70.58	21.28	19.71	69.67	22.64	123.67	128.00	40.89	28.61	30.81	54.55	49.52ax 53.71eq	56.56[a]
19	119.17	115.18	140.96	147.45	89.43	70.43	132.69	125.41	59.53	20.86	123.83	128.93	41.57	38.18	32.59	46.35	40.56	64.15[b] 14.84[c]
20	119.80	117.79	140.17	147.86	91.20	66.49	133.47	128.46	59.02	20.66	127.87	131.43	42.96	40.90	35.93	46.60	43.20	72.45[d] 22.38[e] 22.55[e]
21	118.19	119.31	139.51	148.39	89.40	70.48	132.66	125.45	59.54	20.97	124.27	129.24	41.52	38.19	30.60	46.38	40.56	71.11[d] 22.09[e] 22.13[e]
22	119.53	114.46	141.98	145.68	90.57	70.32	130.04	130.63	59.57	20.56	123.94	128.63	41.85	37.35	33.03	46.94	40.53	56.20[a]
23	118.42	121.94	135.13	149.53	89.38	70.59	132.48	125.64	59.38	24.31	126.50	128.91	42.87	35.00	32.85	45.90	40.06	
Codeine	118.35	113.21	141.23	147.19	91.96	66.44	133.33	128.37	57.86	20.07	127.37	131.14	42.91	40.52	35.47	45.86	42.74	56.01[a]
Dihydro-codeine	117.97	113.96	140.81	146.99	90.34	65.93	25.82	19.36	58.85	19.65	127.13	130.34	41.85	38.37	37.18	46.00	42.73	56.23[a]
Dihydro-morphine	117.97	116.67	137.95	146.01	89.93	66.09	25.59	19.47	58.90	19.59	125.18	130.06	42.00	38.18	37.23	46.02	42.75	
Morphine	118.47	116.25	138.40	146.19	91.42	66.34	133.34	128.40	57.97	20.06	125.46	130.94	42.91	40.56	35.48	45.95	42.77	

[a] OCH_3.
[b] OCH_2CH_3.
[c] OCH_2CH_3.
[d] $OCH(CH_3)_2$.
[e] $OCH(CH_3)_2$.

nation to these strong hydrogen–bond interactions. Significant differences were not found in the spectra of the quaternary compounds (**11, 12**) suggesting that methylation of the nitrogen does not influence ring conformation or intramolecular interactions.

3. Experimental

3.1. Materials and methods

The reagents were purchased from Aldrich and used without further purification. Solvents were freshly distilled prior to use. Organic solutions were dried over anh. Na_2SO_4. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) spectra: 600 MHz Varian VNMRS spectrometer in DMSO-d_6 solutions; δ in ppm rel. to Me_4Si as internal standard. Melting points were taken on a Stuart SMP-3 apparatus. Mass spectra: Agilent 6410 Triple Quad instrument using electrospray ionization (ESI) and negative polarity. UV, CD and ORD spectra: Jasco J-810 instrument, pH 7 aqueous solution, concentrations $2.5–2.8 \times 10^{-4}$ mol dm^{-3}. Structures of the synthesized compounds, results for TLC analyses, melting points, yields and MS data are shown separately (Table 3).

3.2. Synthesis of morphine-6-O-sulfate (*4*)

Morphine hydrochloride (**1**) (1.70 g, 5.28 mmol) was dissolved in water by stirring at room temperature and sodium hydrogen carbonate (17.00 g) was added. To the resultant suspension 4×1.5 ml acetic anhydride was added in 10 min intervals, and then stirred for another 15 min. The aqueous phase was then extracted with chloroform and dried. After evaporation of chloroform the pale yellow oil product **2** was dissolved in 10 ml dry pyridine. To the resultant solution pyridine-SO_3 complex (1.43 g, 9.00 mmol) was added and stirred for 3.5 h at 60 °C. The crude **3** was crystallized from hot water. **3** was dissolved in a mixture of 30 ml methanol and 5 ml 10% aqueous NaOH and stirred for 1 h at room temperature. The solution was neutralized with concentrated acetic acid and the methanol was then evaporated under reduced pressure. The crude product was filtered and washed with methanol and crystallized from hot water to yield **4** (colorless crystals).

3.3. Synthesis of morphine-3-O-sulfate (*8*)

3,6-*O*-diacetyl-morphine (**5**) (2.00 g, 5.41 mmol) was dissolved in ethanol. To the resultant solution 0.38 g (5.46 mmol) hydroxylamine-hydrochloride was added and stirred for 45 min at 60 °C. After the addition of water, the ethanol was evaporated under reduced pressure. The residue was basified (pH 9) with concentrated NH_4OH solution. The aqueous phase was extracted with chloroform and the organic phase was dried. The chloroform was then evaporated under reduced pressure to give 1.55 g **6**. **6** was then sulfated using the method described for **4**. The product was crystallized from hot water followed by hydrolysis of the 6-*O*-acetyl

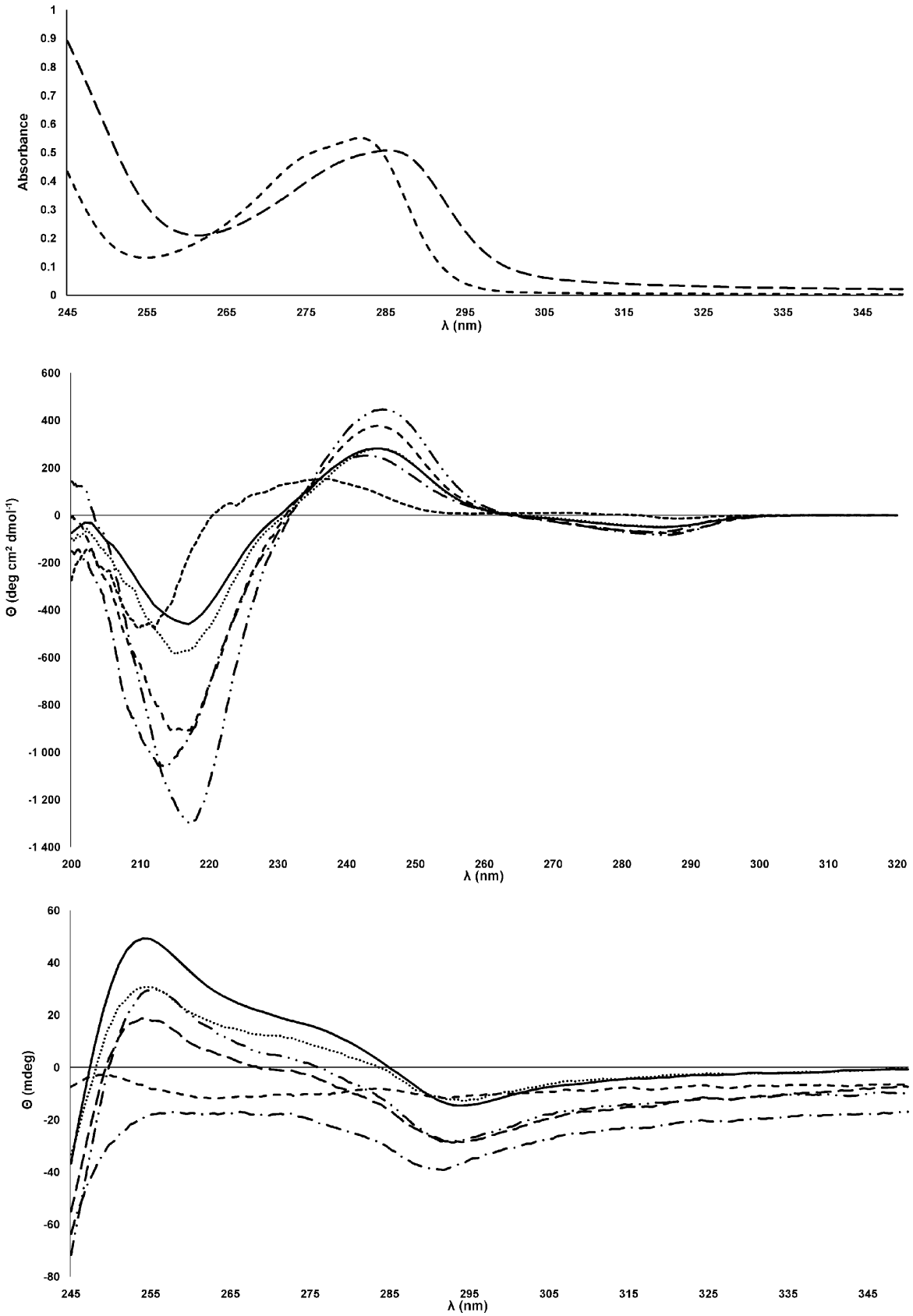
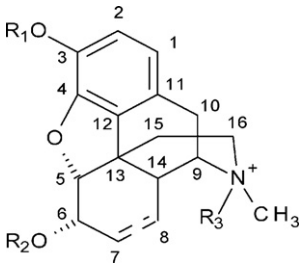


Fig. 1. UV (top), CD (middle) and ORD (bottom) spectra of selected sulfate esters. Solid line: **1**, long dash: **4**, short dash: **8**, dash-dot-dot-dash: **15**, dash-dot-dash: **22**, dotted line: **23**.

Table 3
Structure and physicochemical data of the synthesized compounds.

Compound		R_1	R_2	R_3	C7–C8 bond	Total yield [%]	M.p. [°C]	R_f[c]	ESI-MS
	**4**	H	SO_2O^-	H	Double	32.4[a]	>256[b]	0.34	365
	8	SO_2O^-	H	H	Double	58.4[a]	>280[b]	0.31	365
	9	H	SO_2O^-	H	Single	51.7[a]	>300[b]	0.32	367
	10	SO_2O^-	H	H	Single	56.9[a]	288–292[b]	0.33	367
	11	H	SO_2O^-	CH_3	Double	69.7	>284[b]	0.23	n/a[d]
	12	SO_2O^-	H	CH_3	Double	49.5	>285[b]	0.15	n/a[d]
	13	H	SO_2O^-	CH_3	Single	66.4	>300[b]	0.18	n/a[d]
	14	SO_2O^-	H	CH_3	Single	63.0	>300[b]	0.18	n/a[d]
	15	CH_3	SO_2O^-	H	Double	36.6[a]	239–241[b]	0.24	379
	16	CH_3	SO_2O^-	H	Single	66.7[a]	270–272[b]	0.23	381
	17	CH_3	SO_2O^-	CH_3	Double	73.5[a]	>285[b]	0.19	n/a[d]
	18	CH_3	SO_2O^-	CH_3	Single	90.5	>273[b]	0.14	n/a[d]
	19	Et	SO_2O^-	H	Double	43.9[a]	>218[b]	0.38	393
	21	^{i}Pr	SO_2O^-	H	Double	48.3[a]	>225[b]	0.39	407
	22	CH_3	SO_2O^- (6β)	H	Double	68.7[a]	242–247[b]	0.25	379
	23	SO_2O^-	SO_2O^-	H	Double	40.3	175–178	0.27	445

[a] Recrystallized yields.
[b] Decomposition.
[c] TLC. Mobile phase: 45:15:10:20 mixture of butan-1-ol, acetone, *cc.* acetic acid, and water, respectively. Stationary phase: *Merck Silica Gel 60 F254.*
[d] Quaternary sulfate esters cannot be detected by ESI-MS.

protecting group by the method described for **4**. The crude product was filtered and washed with methanol and crystallized from hot water to give **8** (colorless crystals).

3.4. Synthesis of morphine-3,6-O-disulfate ammonium salt (*23*)

Morphine (**1**) (0.175 g, 0.612 mmol) was dissolved in 3 ml of dry DMF and cooled to 0 °C. To this solution 0.20 ml (6 eq.) of concentrated sulfuric acid and DCC (1.15 g) were added and stirred for 15 min at 0 °C. The reaction was then basified (pH 9) with 10% NH_4OH and filtered. The filtrate was evaporated *in vacuo* and the residue dissolved (3 ml of DMF) and filtered to remove inorganic residue. To the filtrate 50 ml Et_2O was added and the resulting suspension was filtered to give the ammonium salt of **23** (pale yellow solid).

3.5. Synthesis of codeine-6-O-sulfate (*15*)

Codeine (0.90 g, 3.00 mmol) was sulfated using the method described for **4**. The product slowly precipitated during the reaction time as a white powder. Cold water (10 ml) and chloroform (10 ml) were added to the suspension and was left to stand overnight in the freezer. The precipitate was collected by filtration and washed twice with cold water and crystallized from hot water to give **15** (colorless crystals).

3.6. Synthesis of codeine-methylammonium-6-O-sulfate (*17*)

Codeine (2.00 g, 6.68 mmol) was dissolved in 20 ml dry methanol. To the resulting solution 0.44 ml methyl iodide was added and stirred for 4 h at 40 °C. The mixture was filtered and the product washed with acetone to give 2.72 g (6.14 mmol) codeine–methylammonium–iodide (mp. 260–264 °C). 0.88 g (2.00 mmol) of codeine–methylammonium–iodide was dissolved in a mixture of 20 ml dry pyridine and 10 ml of dry DMF and stirred for 3 h at 60 °C after the addition of 0.96 g (6.00 mmol) pyridine–SO_3 complex. The mixture was filtered and the product washed with chloroform to give **17** (white semi-crystalline powder).

3.7. Synthesis of 3-O-isopropylmorphine-6-O-sulfate (*21*)

Sodium (0.25 g, 10.90 mmol) was dissolved in 50 ml ethanol. To the resultant solution morphine (**1**) (2.7 g, 9.00 mmol) and 2-iodopropane (1.3 ml) were added and stirred for 6 h at 60 °C. The ethanol was evaporated under reduced pressure and to the residue was added 50 ml water and 5 ml 10% aqueous NaOH solution followed by extraction with chloroform. The organic phase was dried and the chloroform evaporated under reduced pressure to yield 2.00 g of 3-*O*-isopropylmorphine (**20**) (oil). **20** was then sulfated as described for **4**. The crude product was crystallized from hot water to yield **21** (pale yellow crystals).

4. Conclusion

In conclusion, we synthesized a systematic series of sulfate monoesters of morphine and its derivatives and also, the diester morphine-3,6-*O*-disulfate. For monosubstitution, pyridine–SO_3 complex was used with moderate yields. In the case of the diesters the sulfations were performed with DCC/*cc.* H_2SO_4 reagent. Detailed ^{1}H- and ^{13}C-NMR and CD/ORD analyses are presented that can serve as a basis for further physicochemical investigations or as a tool for the identification of various sulfate esters in biological samples. Most of the synthesized sulfate esters have the potential of selective peripheral analgesic activity.

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