Suramin Analogues with a 2-Phenylbenzimidazole Moiety as Partial Structure; Potential anti HIV- and Angiostatic Drugs, 2[1)]:

Sulfanilic Acid-, Benzenedisulfonic Acid-, and Naphthalenetrisulfonic Acid Analogues☆

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Key Words: *Suramin; benzimidazole analogues synthesis; in vitro anti HIV activity; in vitro antitumor activity; angiogenesis inhibition*

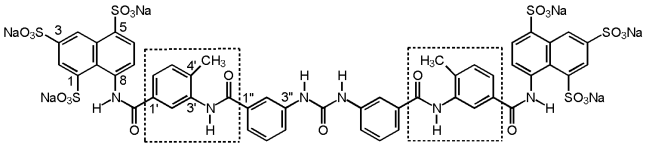
Summary

The synthesis of suramin analogues bearing a 2-phenyl-benzimidazole moiety is described. Aminoarene sulfonic acids **2a–e** are acylated with 3,4-dinitrobenzoyl chloride **3** yielding the amides **4a–e** which are hydrogenated to the corresponding diamines **5a–e**. These are treated with 3-nitrobenzaldehyde, yielding the azomethines **7a–e** and their isomers **8a–e** and **9a–e**. Key step in the synthesis of the target compounds **12a–e** is the oxidation of the azomethines with oxygen to the benzimidazoles **10a–e**. These are hydrogenated to the amines **11a–e** reacting with phosgene to yield the symmetric ureas **12a–e**. Results of the anti-HIV, cytostatic, and antiangiogenic screening are presented.

Introduction

Suramin **1** was developed by Bayer as a drug for the treatment of African sleeping sickness, a trypanosome infection, and has been used for the treatment of this disease since 1920 [1, 2]. In 1947, its activity against Onchocerca volvulus, the causative agent of Onchocerciasis (river blindness), was reported [3]. To this day, it is the only approved macrofilaricidal drug available for the treatment of Onchocerciasis. The interest in new macrofilaricidal agents has declined during the last decade due to the great success of the WHO Onchocerciasis Control Program (OCP) since 1987, when large scale prophylactic Ivermectine treatment was introduced [4]. However, further biological activities of suramin stimulated the continuation of the synthetic work in the suramin field. Suramin is a potent inhibitor of the reverse transcriptase of retro-viruses [5] and has an anti HIV activity [1]. Because of severe toxic side effects, however, suramin is not recommended for the treatment of AIDS [1]. Recently, it has been shown that suramin is an anticancer drug with a unique mode of action, it inhibits tumor growth factors [6]. Therefore, suramin has become an interesting lead for the development of new cytostatic agents.

The replacement of benzene rings by heterocyles in pharmacologically active compounds is a common method applied in investigations of structure activity relationships. Suramin analogues in which benzene rings have been replaced by furan [7] or pyrrole [8] rings are described in the literature. Here we describe the synthesis of suramin analogues in which the enframed parts of the suramin molecule (see Fig. 1) have been replaced by a 2-phenylbenzimidazole moiety. The rationale for this insertion was the fact that 2-phenylbenzimidazole was the lead compound for the development of a series of potent anthelmintics [9]. Originally, our main interest in the suramin field was the discovery of new macrofilaricidal compounds. Therefore, we tried to combine structural elements of different anthelmintic agents in one molecule. Furthermore, an anti-HIV activity of suramin and its analogues seems to require a flat and rigid structure [10]. The replacement of the enframed parts of the suramin molecule by 2-phenylbenzimidazole will lead to compounds with molecular dimensions that are very similar to those of the suramin molecule, but they will have an increased rigidity. With the aim of studying the influence of this replacement on the biological activities of suramin, we synthesized a series of suramin analogues replacing the enframed parts of **1** by benzimidazole and varying the trisulfonaphthyl residues of suramin [11, 12]. This report is the continuation of our research on the synthesis of suramin analogues with 2-phenylbenzimidazole as partial structure [13].



Suramin 1

Figure 1. Suramin.

Chemistry

The four step synthetic pathway to the targets **12a–e** is outlined in Scheme 1. Aqueous solutions of the aminoarenesulfonic acids **2a–e** were treated at pH 4.5 with a solution of 3,4-dinitrobenzoyl chloride (**3**) [14] in toluene giving the dinitrobenzamides **4a–e** in high yields. **4a–e** were catalytically hydrogenated to the corresponding diamines **5a–e** . Using palladium catalysts, deep-red reaction products were obtained. The TLC showed several coloured by-products, presumably azo and azoxy derivatives. The hydrogenation conditions have been systematically varied. Best results were obtained using PtO_2 as the catalyst and a mixture of metha-

[1)] Part 1 of this series see ref.[13].

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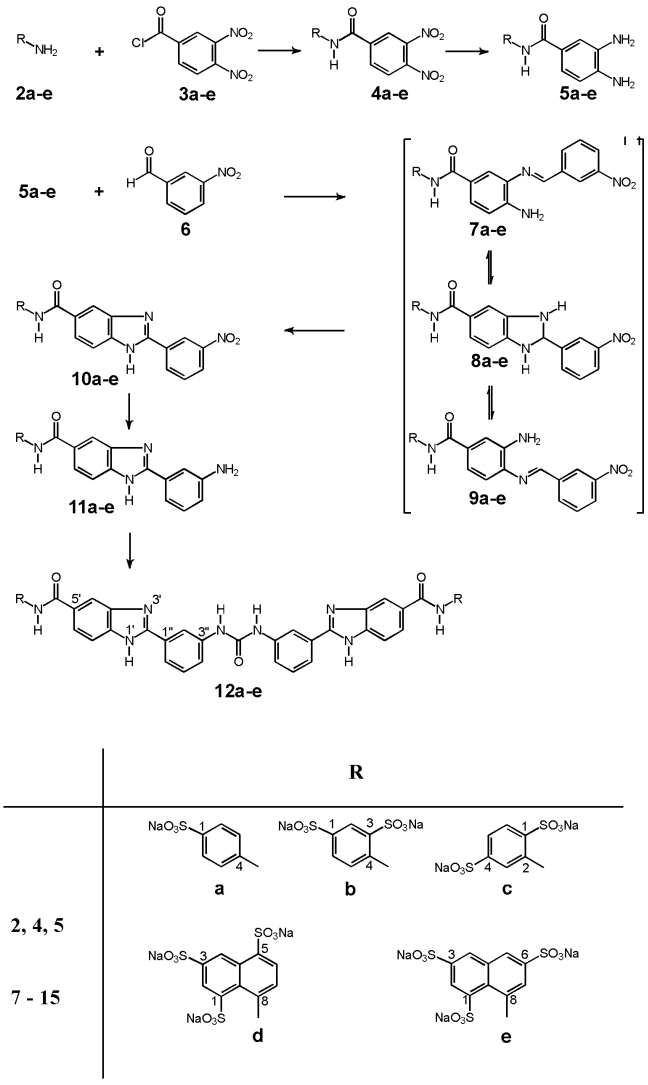
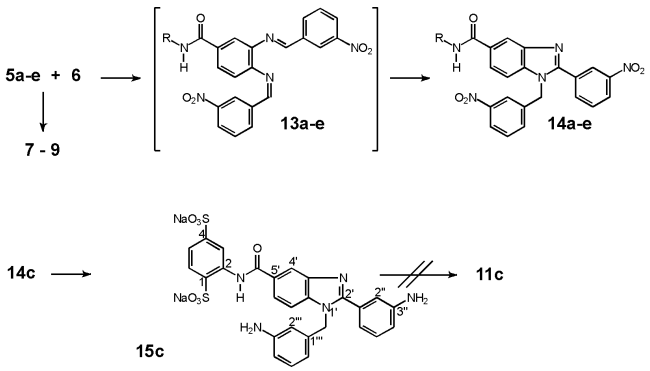


Scheme 1. Synthesis of the new benzene sulfonic, benzene disulfonic, and naphthalenetrisulfonic acid analogues of suramin.

nol/water as the solvent. **5a–e** are very sensitive towards oxidation by air. Therefore, they have not been isolated but were immediately treated with 3-nitrobenzaldehyde (**6**). When equimolar amounts of **5a–e** and **6** were used, **5a–e** could be detected by TLC even after a reaction time of 12 h at room temperature (RT). Therefore, an excess of **6** was used. It can be assumed that the regioisomeric azomethines **7a–e** and **9a–e** are formed together with the dihydrobenzimidazole derivatives **8a–e**. No attempts were made to isolate one of these isomers. The mixture of isomers was directly oxidized with oxygen [15] to the corresponding benzimidazoles **10a–e** according to our recently developed strategy [13].

By-products in this reaction were the 1-(3-nitrobenzyl)-benzimidazole derivatives **14a–e** resulting from the intermediate formation of the bis(benzylidenimino) derivatives **13a–e** during the reaction of **5** with **6** (Scheme 2). **14a–e** could be separated from **10a–e** by recrystallisation.

Catalytic hydrogenation (Pd/C) of the nitro compounds **10a–e** gave the corresponding aromatic amines **11a–e**. *N*-Benzyl groups can be smoothly removed by catalytic hydro-



Scheme 2. Formation of the 1-(3-nitrobenzyl)benzimidazole derivatives **14 a–e** and their reduction to **15 a–e**.

genolysis [16]. Therefore, we expected that catalytic hydrogenation of **14a–e** will lead to a reduction of the nitro groups and a hydrogenolytic debenzylation yielding **11a–e** (Scheme 2). This reaction was intensely investigated with **14c** using different solvents and catalysts (Pd, Pt). Surprisingly, no debenzylation could be observed. **15c** was the main hydrogenation product of **14c**.

Treatment of aqueous solutions of the arylamines **11b–e** with a solution of phosgene in toluene at pH 4.5 resulted in the target ureas **12b–e**. The sulfanilic acid derivative **11a** was not soluble in water at this pH. Therefore, the reaction with **11a** was carried out in suspension according to previous experiments [13] yielding **12a**.

Spectroscopic data collected for the synthesized compounds were in agreement with the proposed structures. However, in the ^{13}C-NMR spectra of the benzimidazole derivatives **10a–e** no resonances were detected for the carbons 3a, 4, 7, and 7a of the heterocycle. This could be explained by the tautomeric character of the imidazole. As expected, addition of one equivalent of trifluoroacetic acid to the sample resulted in the occurrence of the previously missing ^{13}C resonances.

Benzimidazoles are amphoteric compounds. The NH-acidic character of benzimidazoles allows to determine the complete conversion of **7–9** to **10** by titration of **10** in DMF with 0.1 N aqueous NaOH [17]. The purity of all compounds screened biologically was determined by HPLC using our recently published method for the determination of suramin [18].

Biological Screening

The in-vitro anti-HIV screening and the cytostatic screening were carried out in the Department of Health and Human Services, National Cancer Institute, Developmental Therapeutics Program, Bethesda, Maryland 20892, USA. The anti-angiogenic screening was done at the VA Medical Center and Departments of Obstetrics/Gynecology and Physiology, University of Kentucky College of Medicine, Lexington, KY, USA. The results of the biological screening are shown in Tables 1 – 3. In these tables the new compounds are cited according to their numbers from Scheme 1 and in parentheses with their NF codes (N stands for Nickel and F for Filariasis; originally our main interest in the suramin field was the

finding of new filaricidal compounds). This is for comparison purposes because in previous publications [22, 23, 25] our suramin analogues have NF codes.

Anti HIV Screening: The sreening method is described in detail in ref. [19]. Briefly, the test compound is dissolved in DMSO. The DMSO solution is diluted with culture medium. T4 lymphocytes (CEM cell line) are added and after a brief interval HIV-1 is added. Uninfected cells with the compound serve as toxicity controls, and uninfected cells without the compound serve as basic controls. The results of the test are described by the expressions IC50, EC50, TI50. Eight different concentrations of the test compound are used for the calculations of IC50 and EC50. The highest concentration of the test compounds in this screening system is normally 100 µM. IC50 >100µM means: no cell toxicity observed at this concentration. The results of the anti HIV screening are shown in Table 1.

Table 1. Anti HIV activity of the benzimidazole analogues of suramin.

Compound	EC50 µM	IC50 µM	TI50
Suramin	33.5	>174	>5.2
	39.0	115	2.9
	34.6	>174	>5.0
	35.6	>174	>4.9
12a	3.4	>150	>45
(NF503)	8.6	>150	>17
	58.5		
	60.0		
12d	37.0	64.8	1.8
(NF506)	29.9	60.9	2.0
	29.0	72.7	2.5
	30.0	82.4	2.8
17	2.2	>1000	>457
(NF417)	23.2	>200	>8.6
	51.3	>1000	>19.5
	81.4	>200	>2.5
	147.0	>200	>1.4

IC50 µM: Toxicity, concentration of the test compound which decreases the cell growth of the uninfected cell culture to 50% of the untreated cell culture.
EC50 µM: Efficiency, concentration of the test compound which increases the cell growth of the infected cell culture to 50% of the uninfected, untreated cell culture
TI50: Therapeutic index, IC50/EC50

Cytostatic Screening: In the NCI program, the cytostatic activity of the test compounds against 60 tumor cell lines is investigated. The cell cultures are incubated with five different concentrations of the test compounds. The highest concentration tested is normally 100 µM. The results of the test are described by the expressions GI50, and TGI (Total Growth Inhibition). None of the compounds described in this paper was able to totally inhibit tumor cell growth at the highest tested concentration. The results of the NCI cytostatic screening are shown in Table 2.

Table 2. In vitro cytostatic activity of the benzimidazole analogues of suramin.

Cmpnd	Tumor cell line	Type of cancer	–log(GI50) [a] NF	Suramin	S [b]
12a					
(NF503)	K-562	Leukemia	4.07	<4.00	>1.17
12d	MCF7/ADR-RES	Breast C.	4.10	<4.00	>1.26
(NF506)	COLO 205	Colon C.	4.18	<4.00	1.51
	KM 12	Colon C.	4.40	4.04	2.29
	SW-620	Colon C.	4.16	4.11	1.12
	CCRF-CEM	Leukemia	4.24	<4.00	>1.74
	HL-60 (TB)	Leukemia	4.12	4.09	1.07
	LOX IMVI	Melanoma	4.27	4.13	1.38
	ACHN	Renal C.	4.11	<4.00	1.29
	UO-31	Renal C.	4.13	4.59	0.35
17	MDA-MB-231/ATCC	Breast C.	4.33	<4.00	>2.14
(NF417)	CCRF-CEM	Leukemia	4.53	<4.00	>3.39
	HL-60 (TB)	Leukemia	4.61	4.09	3.31
	SR	Leukemia	4.71	4.16	3.55
	M14	Melanoma	4.34	4.26	1.20
	IGRO VI	Ovarian C.	4.35	4.39	2.19

[a] GI50: Growth Inhibition, concentration M of the test compound which decreases the tumor cell growth to 50% of the untreated tumor cell culture.
[b] S: Selectivity factor GI50(Suramin)/GI50(NF), values > 1 indicate that the NF-compound is more active than suramin.

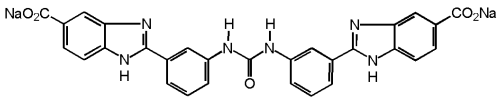
Antiangiogenic screening: Suramin has a unique mode of cytostatic action; it inhibits tumor growth factors [6]. The inhibition of the angiogenesis growth factor is a main target for the development of new anti tumor drugs. The antiangiogenic activity of suramin and of suramin analogues is described in the literature [20–22]. The antiangiogenic activity was investigated in the chick egg chorioallantoic membrane (CAM) assay. The method is described in detail in the literature [20, 22]. Briefly, fertile chick eggs are incubated for 72 h at 37 °C. After 72 h the egg shells are broken and the egg contents are placed in petri dishes. Each compound to be tested is dissolved in an aqueous 0.45% solution of methylcellulose. A 10 µl aliquot of this solution is air dried on a

Table 3. Inhibition of angiogenesis by the benzimidazole analogues of suramin.

Compound	# Embryos	% Inhibition	ID50 nmol/disk
Suramin	67	64	75
12c			
(NF504)	23	33	60
12d			
(NF506)	20	58	70
12e			
(NF507)	21	60	65

Embryos: number of eggs tested
% Inhibition: percentage of the investigated eggs in which an inhibition of angiogenesis was observed on treatment with approximatively 70 nmol/disk of the compound
ID50: dose in nmol/disk that induces 50 % inhibition of angiogenesis in the CAM assay

Teflon-coated metal tray forming a disk around 2 mm diameter. This disk is implanted on the outer third of six-day CAM where capillaries are intensively growing. The zone around the methylcellulose disk is examined 48 h after implantation. A positive inhibition of angiogenesis is indicated by an avascular area of ≥ 4 mm. Standard dose of the test compound is 70 nmol/disk. The results of the anti angiogenic screening are shown in Table 3.



17 (NF417)

Figure 2. Compound **17 (NF417)**.

Discussion

Anti HIV activity: Simian reverse transcriptase SIVagmTYO-7 RT was inhibited by **12d** with an IC50 of 0.5 μM [23]. The batch of **12d** [11] used in this experiment was not very pure. This high inhibition of SIV-RT could later not be reproduced with a very pure sample (HPLC purity > 99 %) of **12d (NF506)** [12]. This disappointing result could be due to the high non-selective binding of **12d (NF506)** to proteins, e.g. to serum albumin [24]. The repeated investigation of **12d (NF506)** was done with a different batch of the enzyme. The purity of the enzyme can have an important influence on the test result.

Similar confusing results were obtained with compounds **12a (NF503)** and **17 (NF417)** [13] in the NCI HIV test shown in Table 1. The high activities and selectivities of both compounds could not be reproduced when the experiments were repeated after several months. But, in contrast to the experiments with SIV-RT, all results cited in Table 1 were done with the same batches of very pure compounds. With suramin and with **12d (NF506)**, the results were reproducible when the experiments were repeated after some months. **12d (NF506)** seems to be slightly more active but also more toxic than suramin. Therefore, **12d (NF506)** has no advantage compared with suramin. **12d (NF506)** is like suramin a hexasulfonate. It is highly soluble in water. The results of the four different tests do not vary significantly. In contrast, **12a (NF503)** and **17 (NF417)** have a very low solubility in water. In the NCI screening, the compounds are dissolved in DMSO. These solutions are added to the aqueous medium of the cell cultures. We assume that the compounds precipitate partially in the medium. Only the dissolved compounds can lead to a biological activity. The consequence of this (certainly not reproducible) precipitation will be a strong variation of the results. The activity of the dicarboxylic acid **17 (NF417)** [13] is quite interesting. In all earlier screenings of suramin analogues, the activities were completely lost when sulfonate substituents were replaced by carboxylate substituents [26, 27].

Cytostatic activity: In Table 2, the cytostatic activities of 3 new suramin analogues are presented. Neither suramin nor any one of the investigated suramin analogues was able to inhibit totally the tumor cell growth at the highest tested concentration. All cell lines against which the compounds showed an activity at concentrations ≤100 μM are shown. **12a (NF503)**, a disulfonate shows a marginal activity against only one leukemia cell line (K-562). In contrast, **12d (NF506)** which is a close analogue of suramin inhibits 9 cell lines. However, the activities are also marginal. The activities are compared with the activity of suramin against these cell lines. Suramin is inactive against 4 cell lines at the highest investigated concentration (100 μM). Against 5 cell lines, **12d (NF506)** and suramin show comparable low activities. Here again, the activity of the dicarboxylic acid analogue **17 (NF417)** is of special interest. It is more active than the sulfanilic acid analogue **12a (NF503)**. Against 7 cell lines, it shows an activity comparable or slightly higher than that of suramin. However, the spectrum of activity of **17 (NF417)** differs markedly from that of suramin. It shows the highest activity against three leukemia cell lines against which suramin and **12a (NF503)** are completely inactive.

Inhibition of angiogenesis: Suramin inhibits tumor growth factors [6]. The most interesting target of suramin analogues is the inhibition of angiogenesis. This activity can not be tested in a cell culture. The three benzimidazole analogues **12c (NF504)**, **12d (NF506)**, and **12e (NF507)** show activities comparable to that of suramin. The tetrasulfonate **12c (NF504)** has the lowest ID_{50}.

Conclusion

The title compounds, suramin analogues with 2-phenylbenzimidazole as partial structure, show biological activities comparable to those shown by suramin in the cited test systems. These results differ significantly from those observed with trypanosomes. Very small variations of the suramin structure, e.g. the substitution of the two methyl groups by hydrogen or other substituents, lead to a nearly complete loss of trypanocidal activity [27].

Acknowledgment

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Experimental

The benzenedisulfonic acids **2b**,**c** were a gift from Hoechst, the naphthalenetrisulfonic acids **2d–e** were a gift from Bayer. Oxidations were performed in: Laborautoclave HR 500 (Berghof) with heating equipment HT 20 (Horst) and magnetic stirring equipment. pH-Stat equipment: Metrohm 718 STAT Titrino. Titrations: Metrohm Titroprozessor 672; determination of the equivalent mass: about 0.1 mmol of the compound was dissolved in 5.0 ml of DMF and titrated with aqueous 0.1 N NaOH (glass electrode). TLC: Merck aluminum sheets with silica gel $60F_{254}$, solvent 2-propanol/NH_3 25%, 5:2. NMR (solvent as internal standard): Varian XL 300, chemical shifts are given in ppm; pt: pseudotriplet; coupling constants are given in Hertz (Hz). FAB-MS: Kratos concept 1H. IR: (KBr) Perkin-Elmer 1420. UV/VIS: Spectrophotometer HP 8451 A. The purity of all suramin analogues described in this paper was determined by the HPLC method described in ref. [18].

Experimental details are described only for the synthesis of the naphthalene-1,3,6-trisulfonic acid derivative **12e**. The sulfanilic acid (**a**), benzene-1,3-disulfonic acid (**b**), benzene-1,4-disulfonic acid (**c**) and naphthalene-1,3,5-trisulfonic acid (**d**) derivatives were prepared in a similar manner starting from **2a**, **2b**, **2c**, or **2d**, respectively instead of **2e** (see Table 4).

Table 4. Synthetic and analytical data of the sulfanilic acid (**4a–12a**), benzene disulfonic acid (**4b,c–12b,c**), and naphthalenetrisulfonic acid (**4d,e–12d,e**) derivatives .

Compd.	Starting material	Method	yield %	TLC R_f	Formula	Molecular mass calcd.	found MS	found titration[a)]	purity HPLC
4a	**2a, 3**	A	69	0.68	$C_{13}H_8N_3NaO_8S$	389.27	388(M-H)		
4b	**2b, 3**	A	72	0.52	$C_{13}H_9N_3O_{11}S_2$	447.36	446(M-H)		
4c	**2c, 3**	A	49	0.58	$C_{13}H_7N_3Na_2O_{10}S_2$	491.32			
					$C_{13}H_{10}N_3O_{10}S_2$	447.34	446(M-H)		
4d	**2d, 3**	A	78	0.51	$C_{17}H_{11}N_3O_{14}S_3$	577.48	576(M-H)		
4e	**2e, 3**	A	72	0.55	$C_{17}H_{11}N_3O_{14}S_3$	577.48	576(M-H)		
10a	**7a - 9a**	B	68	0.70	$C_{20}H_{13}N_4NaO_6S$	460.40		463.3	
					$C_{20}H_{14}N_4NaO_6S$	438.42	437(M-H)		
10b	**7b–9b**	B	40	0.56	$C_{20}H_{12}N_4Na_2O_9S_2$	562.44	539(M+Na-2H)	571.6	
10c	**7c–9c**	B	52	0.58	$C_{20}H_{12}N_4Na_2O_9S_2$	562.44	539(M-Na)	565.1	
10d	**7d–9d**	B	36	0.41	$C_{24}H_{13}N_4Na_3O_{12}S_3$	714.55	691(M-Na)		
10e	**7e–9e**	B	39	0.55	$C_{24}H_{13}N_4Na_3O_{12}S_3$	714.55	691(M+H-2Na)	704.7	
11a	**10a**	C	61	0.66	$C_{20}H_{15}N_4NaO_4S$	430.42	429(M-H)	422.5	
11b	**10b**	C	66	0.48	$C_{20}H_{14}N_4Na_2O_7S_2$	532.46	487(M+H-2Na)	527.4	
11c	**10c**	C	49	0.55	$C_{20}H_{14}N_4Na_2O_7S_2$	532.46	487(M+H-2Na)	566.0 [b)]	
11d	**10d**	C	53	0.47	$C_{20}H_{15}N_4Na_3O_{10}S_3$	684.57	661(M-Na)	679.9	
11e	**10e**	C	53	0.47	$C_{20}H_{15}N_4Na_3O_{10}S_3$	684.57	661(M-Na)	679.9	
12a	**11a**	D	51	0.53	$C_{41}H_{28}N_8Na_2O_9S_2$	886.83	885(M-H)	450.6	
12b	**11b**	D	26	0.44	$C_{41}H_{26}N_8Na_4O_{15}S_4$	1090.92	1067(M-Na)	532.4	~100%
12c	**11c**	D	18	0.41	$C_{41}H_{26}N_8Na_4O_{15}S_4$	1090.92	1045(M+H-2Na)	496.8	~95%
12d	**11d**	D	34	0.40	$C_{49}H_{28}N_8Na_6O_{21}S_6$	1395.13	1371(M-Na)		>99%
12e	**11e**	D	36	0.51	$C_{49}H_{28}N_8Na_6O_{21}S_6$	1395.13	1327(M+2H-3Na)	716.7 [b)]	>99%

[a)] titration in DMF with aqueous 0.1 N NaOH

[b)] contains 2 mol H_2O

*A) 8-(3,4-Dinitrobenzamido)-1,3,6-naphthalenetrisulfonic acid trisodium salt (**4e**)*

To a solution of **2e** (21.4 g, 50 mmol) in water (150 ml), 3,4-dinitrobenzoyl chloride (**3**, 23 g, 100 mmol), dissolved in 100 ml of toluene, was added under vigorous stirring. During the reaction time (3 h), a pH of 4.5 was maintained by automatic addition of 1 M $NaHCO_3$. The aqueous phase was separated. The organic phase was washed twice with water. The combined aqueous phases were evaporated in vacuo to dryness and the residue was twice recrystallized from EtOH/H_2O (3 : 1). **4e** was obtained as a yellow powder (23.6 g, 78 %).

TLC: R_f 0.55. IR (cm^{-1}): 3470, 3280, 1670, 1550, 1530, 1390, 1350, 1220, 1185, 1040, 845, 675. UV/VIS (H_2O): λ_{max} (logε): 194 (4.56), 238 (4.48), 300 (3.90). ^{1}H-NMR (300 MHz [d_6]DMSO): δ = 8.14 (d, J = 2.0 Hz, 1H, 5-H), 8.26 (d, J = 2.0 Hz, 1H, 7-H), 8.42 (d, J = 8.5 Hz, 1H, 5′-H), 8.64 (d, J = 2.0 Hz, 1H, 2-H), 8.64 (d, J = 2.0 Hz, 1H, 2′-H), 8.69 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, 6′-H), 8.92 (d, J = 2.0 Hz, 1H, 4-H), 12.85 (s, 1H, -NH-CO-). ^{13}C-NMR (75 MHz [d_6]DMSO): δ = 161,75 (C-9), 144.75 (C-1), 143.38 (C-3), 143.01 (C-4′), 141.49 (C-3′), 141.26 (C-6), 140.60 (C-1′), 134.38 (C-8), 133.75 (C-6′), 132.04 (C-4a), 128.10 (C-4), 126.45 (C-5), 125.77 (C-2), 124.91 (C-5′), 123.63 (C-2′), 122.52 (C-8a), 122.49 (C-3). FAB MS (Glycerol/DMSO) *m/z*: 496 [M-SO_3H]$^-$, 560 [M-OH]$^-$, 576 [M-H]$^-$, 668 [M+Glyc-H]$^-$, 690 [M+Glyc+Na-2H]$^-$, 760 [M+2Glyc-H]$^-$, 852 [M+3Glyc-H]$^-$, 944 [M+4Glyc-H]$^-$.

$C_{17}H_8N_3Na_3O_{14}S_3$ (643.42), $C_{17}H_{11}N_3O_{14}S_3$ (577.48).

In a similar way, compounds **4a–d** were synthesized; for analytical data see Table 4.

*B) 8-[2-(3-Nitrophenyl)benzimidazole-5-carboxamido]-1,3,6-naphthalenetrisulfonic acid trisodium salt (**10e**)*

A solution of **4e** (12.8 g, 20 mmol) in MeOH (300 ml) and H_2O (200 ml) was hydrogenated at a hydrogen pressure of 5 bar with PtO_2 as the catalyst. After the hydrogen absorption had come to an end, the reaction mixture was mixed immediately with a solution of 3-nitrobenzaldehyde (**6**, 4.5 g, 30 mmol) in MeOH (60 ml). After 10 min the mixture was filtrated. The filtrate was stirred for 12 h at RT, evaporated to a volume of 100 ml, and oxidized in an autoclave at 120 °C for 12 h under an oxygen pressure of 6 bar. The reaction mixture was evaporated to dryness and the residue was recrys-

tallized from MeOH/H_2O (1:1) yielding 6.5 g (39 %) of **10e** as a beige powder.

TLC: R_f 0.45. Titration: equiv. mass calcd. 714.6; found 704.6. IR (cm^{-1}): 3460, 1660, 1635, 1535, 1390, 1360, 1220, 1200, 1150, 715, 680. UV/VIS (H_2O): λ_{max} (logε): 192 (4.36), 240 (4.75) 316 (4.47). ^{1}H-NMR (300 MHz [d_6]DMSO): δ = 7.91 (dd, *J* = 8.7 Hz, *J* = 0.5 Hz, 1H, 7′-H), 7.99 (pt, *J* = 8.2 Hz, 1H, 5″-H), 8.01 (d, *J* = 2.0 Hz, 1H, 5-H), 8.17 (d, *J* = 1.8 Hz, 1H, 7-H), 8.27 (dd, *J* = 8.5 Hz, *J* = 1.8 Hz, 1H, 6′-H), 8.48 (ddd, *J* = 8.2 Hz, *J* = 2.0 Hz, *J* = 1.0 Hz, 1H, 6″-H), 8.59 (d, *J* = 2.0 Hz, 1H, 2-H), 8.63 (d, *J* = 1.8 Hz, 1H, 4-H), 8.65 (ddd, *J* = 8.2 Hz, *J* = 2.0 Hz, *J* = 1.0 Hz, 1H, 4″-H), 8.8 (dd, *J* = 1.8 Hz, J = 0.5 Hz, 1H, 4′-H), 9.13 (pt, *J* = 2.0 Hz, 1H, 2″-H), 12.75 (s, 1H, -NH-CO-). ^{13}C-NMR (75 MHz [d_6]DMSO): δ = 164.48 (C-9), 149.04 (C-2′), 148.09 (C-3″), 144.71 (C-1), 143.26 (C-3), 141.45 (C-6), 135.85 (C-3a′), 134.34 (C-8), 133.62 (C-7a′), 133.39 (C-6″), 133.01 (C-1″), 132.63 (C-4a), 131.01 (C-5″), 127.85 (C-4), 126.67 (C-5′), 126.39 (C-2″), 126.12 (C-5), 125.53 (C-2), 122.62 (C-7), 122.31 (C-8a), 122.23 (C-4″), 121.94 (C-6′), 114.43 (C-4′), 114.07 (C-7′). FAB MS (Glycerol/DMSO) *m/z*: 567 [M+2H-2Na-SO_3Na]$^-$, 589 [M+H-Na-SO_3Na]$^-$, 631 [M+3H-3Na-OH]$^-$, 647 [M+2H-3Na]$^-$, 669 [M+H-2Na]$^-$.
$C_{24}H_{13}N_4Na_3O_{13}S_3$ (714.55).

In a similar way, compounds **10a–d** were synthesized; for analytical data see Table 4.

*C) 8-[2-(3-Aminophenyl)benzimidazole-5-carboxamido]-1,3,6-naphthalenetrisulfonic acid trisodium salt (**11e**)*

A suspension of **10e** (10 g, 14 mmol) in MeOH (200 ml) and H_2O (200 ml) was hydrogenated at a hydrogen pressure of 5 bar with Pd/C (10% Pd, 200 mg) as the catalyst. **10e** dissolved during the reaction course. The reaction mixture was filtrated, and the filtrate evaporated in vacuo to dryness. Recrystallization in EtOH/H_2O (4:3) gave 5.0 g (52 %) of **11e** as a yellow-green powder.

TLC: R_f 0.77. Titration: equiv. mass calcd. 684.6; found 733.7. IR (cm^{-1}): 3440, 3060, 1655, 1625, 1590, 1565, 1545, 1485, 1385, 1350, 1220, 1175, 1035, 715, 670. UV/VIS (H_2O): λ_{max} (logε): 236 (4.83), 316 (4.47). ^{1}H-NMR (300 MHz [d_6]DMSO): δ = 7.14 (ddd, *J* = 8.3 Hz, *J* = 2.3 Hz, *J* = 1.2 Hz, 1H, 6″-H), 7.45 (pt, *J* = 8.3 Hz, 1H, 5″-H), 7.58 (m, 1H, 2″-H), 7.59 (m, 1H, 4″-H), 7.85 (d, *J* = 8.8 Hz, *J* = 0.5 Hz, 1H, 7′-H), 7.9 (s, 2H, -NH_2), 8.09 (d, *J* = 2.0 Hz, 1H, 5-H), 8.22 (dd, *J* = 8.8 Hz, *J* = 1.8 Hz, 1H, 6′-H), 8.24 (d, *J* = 2.0 Hz, 1H, 7-H), 8.50 (d, *J* = 2.0 Hz, 1H, 2-H), 8.58 (dd, *J* = 1.8 Hz, *J* = 0.8 Hz, 1H, 4′-H), 8.63 (d, *J* = 2.0 Hz, 1H, 4-H), 12.75 (s, 1H, -NH-CO-). ^{13}C-NMR (75 MHz [d_6]DMSO): δ = 164.45 (C-9), 150.97 (C-2′), 144.66 (C-1), 144.40 (C-3″), 143.22 (C-3), 141.44 (C-6), 134.73 (C-3a′), 134.35 (C-8), 133.02 (C-7a′), 132.68 (C-1″), 132.60 (C-4a), 130.24 (C-5″), 127.90 (C-4), 126.11 (C-5), 125.46 (C-2), 124.78 (C-5′), 122.68 (C-7), 122.36 (C-8a), 122.01 (C-6′), 120.96 (C-6″), 118.60 (C-4″), 114.92 (C-4′), 114.05 (C-7′), 113.55 (C-2″). FAB MS (Glycerol/DMSO) *m/z*: 617 [M+2H-3Na]$^-$.
$C_{24}H_{15}N_4Na_3O_{10}S_3$ (684.57).

In a similar way, compounds **11a–d** were synthesized; for analytical data see Table 4.

*D) 8,8-[Carbonylbis(imino-3,1-phenylene-(2,5-benzimidazolylene)-carbonyl-imino)]bis-1,3,6-naphthalenetrisulfonic acid hexasodium salt (**12e**)*

A 2 M solution of phosgene in toluene (20 ml, 40 mmol $COCl_2$) was added dropwise over 30 min at room temperature to a vigorously stirred solution of **11e** (2.7 g, 4 mmol) in H_2O (40 ml). During the reaction time, the mixture was maintained at pH 4.5 by automatic addition of 2 N NaOH. Once the addition was over, stirring was continued for 30 min and the pH was adjusted to pH 6 with 2 M NaOH. The reaction mixture was evaporated to dryness and the residue was recrystallized from EtOH/H_2O (4 : 3) giving **11e** as a white powder (2.2 g), containing 2.1 % NaCl as measured by titration, thus the yield of isolated **12e** was 2.15 g (35 %).

TLC: R_f 0.51. Titration: equiv. mass calcd. 697.6; found 716.7. IR (cm^{-1}): 3440, 3080, 1640, 1590, 1540, 1480, 1450, 1295, 1200, 1140, 1040, 715, 675. UV/VIS (H_2O): λ_{max} (logε): 194 (4.82), 240 (5.01), 316 (4.73). ^{1}H-NMR (300 MHz [d_6]DMSO): δ = 7.56 (pt, *J* = 8.0 Hz, 1H, 5″-H), 7.7 (d, *J* = 8.0 Hz, 1H, 6″-H), 7.84 (d, *J* = 8.0 Hz, 1H, 4″-H), 7.88 (d, *J* = 9.0, 1H, 7′-H), 8.135 (d, *J* = 1.8 Hz, 1H, 5-H), 8.18 (d, *J* = 9.0 Hz, 1H, 6′-H), 8.29 (d, *J* = 2.0 Hz, 1H, 7-H), 8.41 (s, 1H, 2″-H), 8.53 (d, *J* = 1.8 Hz, 1H, 2-H), 8.55 (s, 1H, 4′-H), 8.67 (d, *J* = 2.0 Hz, 1H, 4-H), 9.55 (s, 1H, -NH-CO-), 12.7 (s, 1H, -NH-CO-). ^{13}C-NMR (75 MHz [d_6]DMSO): δ = 151.70 (C-10), 165.55 (C-9), 152.67 (C-2′), 144.36 (C-1), 142.98 (C-3), 141.56 (C-6), 140.48 (C-3″), 136.99 (C-3′), 134.80 (C-8), 134.75 (C-7a′), 133.36 (C-4a), 131.83 (C-1″), 130.29 (C-5″), 128.86 (C-4), 126.56 (C-5), 126.32 (C-5′), 124.98 (C-2), 123.48 (C-7), 123.16 (C-8a), 122.82 (C-6″), 122.61 (C-6′), 121.63 (C-4″), 117.42 (C-2″), 114.88 (C-4′), 114.23 (C-7′). FAB MS (Glycerol/DMSO) *m/z*: 1269 [M+H SO_3Na Na]$^-$, 1327 [M+2H 3Na]$^-$.
$C_{49}H_{28}N_8Na_6O_{21}S_6$ (1395.13).

In a similar way, compounds **12a–d** were synthesized; for analytical data see Table 4.

*E) 3-[1-(3-Nitrobenzyl)-2-(3-nitrophenyl)-benzimidazole-5-carboxamido]-1,4-benzene disulfonic acid disodium salt (**14c**)*

A solution of **4c** (5.0 g, 10.5 mmol) in MeOH (150 ml) and H_2O (60 ml) was hydrogenated and treated with 3-nitrobenzaldehyde (**6**, 2.28 g, 15.1 mmol) according to method **B**. The reaction mixture was oxidized in an autoclave at 120 °C for 12 hr under an oxygen pressure of 6 bar. **10c** (3.1 g, 52 %) precipitated and was isolated by filtration. The filtrate was evaporated to dryness, and the residue was recrystallised from MeOH/H_2O (8 + 1) yielding 1.42 g (19 %) of **14c** as a beige powder.

TLC: R_f 0.58. IR (cm^{-1}): 3485, 1660, 1535, 1410, 1350, 1275, 1220, 1185, 1015, 820, 730. UV/VIS (0.1 N NaOH): λ_{max} (log ε): 298 (4.49). ^{1}H-NMR (300 MHz [d_6]DMSO): δ = 5.9 (s, 2H, -CH_2-), 7.32 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, 6-H), 7.54 (ddd, *J* = 7.8 Hz, *J* = 1.8 Hz, *J* = 1.3 Hz, 1H, 4‴-H), 7.61 (dd, *J* = 8.0 Hz, *J* = 7.8 Hz, 1H, 5‴-H), 7.67 (d, *J* = 8.0 Hz, 1H, 5-H), 7.85 (dd, *J* = 8.5 Hz, *J* = 8.0 Hz, 1H, 5″-H), 7.93 (dd, *J* = 9.0 Hz, *J* = 1.2 Hz, 1H, 6′-H), 7.94 (dd, *J* = 2.5 Hz, *J* = 1.8 Hz, 1H, 2‴-H), 7.96 (d, *J* = 9.0 Hz, 1H, 7′-H), 8.15 (ddd, *J* = 8.0 Hz, *J* = 2.5 Hz, *J* = 1.3 Hz, 1H, 6‴-H), 8.23 (ddd, *J* = 8.0 Hz, *J* = 1.9 Hz, *J* = 1.0 Hz, 1H, 4″-H), 8.26 (d, *J* = 1.2 Hz, 1H, 4′-H), 8.39 (ddd, *J* = 8.5 Hz, *J* = 2.3 Hz, *J* = 1.0 Hz, 1H, 6″-H), 8.51 (dd, *J* = 2.3 Hz, *J* = 1.9 Hz, 1H, 2″-H), 8.79 (d, *J* = 1.6 Hz, 1H, 2-H), 8.83 (d, *J* = 1.9 Hz, 1H, 2-H), 11.45 (s, 1H, -NH-CO-). FAB MS (Glycerol) *m/z*: 572 [M+H-Na-SO_3Na]$^-$, 594 [M-SO_3Na]$^-$, 652 [M+H-2Na]$^-$, 674 [M-Na]$^-$, 744 [M+Gly+H-2Na]$^-$, 766 [M+Gly-Na]$^-$, 788 [M+Gly-H]$^-$.
$C_{27}H_{17}N_5Na_2O_{11}S_2$ (697.57).

*F) 3-[1-(3-Aminobenzyl)-2-(3-aminophenyl)-benzimidazole-5-carboxamido]-1,4-benzene disulfonic acid disodium salt (**15c**)*

14c (1.0 g, 1.29 mmol) was dissolved in MeOH (170 ml) and H_2O (30 ml) and hydrogenated at a hydrogen pressure of 5 bar with Pd/C (10% Pd, 70 mg) as the catalyst. The reaction mixture was filtrated, and the filtrate evaporated *in vacuo* to dryness. Recrystallization of the residue in EtOH/H_2O (8 + 1) gave 0.34 g (41.3 %) of **15c** as a green powder. **11c** was not detectable by TLC.

TLC: R_f 0.55. IR (cm^{-1}): 3420 3060, 1660, 1605, 1575, 1530, 1450, 1400, 1275, 1215, 1185, 1015, 660. UV/VIS (0.1 N NaOH): λ_{max} (logε): 230 (4.68), 304 (4.40). ^{1}H-NMR (300 MHz [d_6]DMSO): δ = 5.0 (s, 4H, 2 × –NH_2), 5.43 (s, 2H, -CH_2-), 6.20 (ddd, *J* = 7.7 Hz, *J* = 1.8 Hz, *J* = 1.1 Hz, 1H, 6‴-H), 6.43 (ddd, *J* = 8.0 Hz, *J* = 2.2 Hz, *J* = 1.0 Hz, 1H, 4‴-H), 6.48 (dd, *J* = 2.2 Hz, *J* = 1.8 Hz, 1H, 2‴-H), 6.76 (ddd, *J* = 8.0 Hz, *J* = 2.3 Hz, *J* = 1.0 Hz, 1H, 6″-H), 6.87 (ddd, *J* = 7.8 Hz, *J* = 1.8 Hz, *J* = 1.0 Hz, 1H, 4″-H), 6.93 (dd, *J* = 8.0 Hz, *J* = 7.7 Hz, 1H, 5‴-H), 7.08 (dd, *J* = 2.3 Hz, *J* = 1.8 Hz, 1H, 2″-H), 7.18 (dd, *J* = 8.0 Hz, *J* = 7.8 Hz, 1H, 5″-H), 7.33 (dd, *J* = 8.3 Hz, *J* = 1.9 Hz, 1H, 6-H), 7.69 (d, *J* = 8.3 Hz, 1H, 5-H), 7.80 (d, *J* = 8.6 Hz, 1H, 7′-H), 7.86 (dd, *J* = 8.6 Hz, *J* = 1.8 Hz, 1H, 6′-H), 8.10 (d, *J* = 1.8 Hz, 1H, 4′-H), 8.83 (d, *J* = 1.9 Hz, 1H, 2-H), 11.3 (s, 1H, -NH-CO-). FAB MS (Glycerol) *m/z*: 512 [M+H-Na-SO_3Na]$^-$, 592 [M+H-Na]$^-$, 614 [M-Na]$^-$, 636 [M-H]$^-$, 706 [M+Gly-Na]$^-$, 798 [M+2Gly-Na]$^-$.
$C_{27}H_{21}N_5Na_2O_7S_2$ (637.61).

References

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