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From mutagenic to non-mutagenic nitroarenes: effect of bulky alkyl substituents on the mutagenic activity of 4-nitrobiphenyl in *Salmonella typhimurium* Part I. Substituents ortho to the nitro group and in 2′-position

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Abstract

Eleven alkyl substituted derivatives of 4-nitrobiphenyl (4NBp) and two corresponding nitroso compounds were synthesised and tested for mutagenic potency in strains TA98 and TA100 of *Salmonella typhimurium*. The mutagenicity of compounds substituted ortho to the nitro group (3-methyl-, 3-ethyl-, 3-*iso*propyl-, 3-*tert*butyl-, 3,5-diethyl-, 3,5-di*iso*propyl-, and 3,5-di*tert*butyl-4NBp) decreased with growing steric demand of the alkyl substituents in both tester strains. The most sterically hindered compounds were non-mutagenic even at highest concentrations. This reduction of mutagenicity is correlated with deviations from the coplanar orientation of the nitro group relative to the aromatic plane. Since a comparable decrease of mutagenicity for the nitroso compounds (4NOBp and 3-*tert*butyl-4NOBp) was not observed, the first reduction step of non-planar nitro groups must be inhibited.

Alkyl groups in the 2′-position of 4NBp (2′-methyl-, 2′-ethyl-, 2′-*iso*propyl-, and 2′,4′,6′-trimethyl-4NBp) also reduced mutagenic activity with increasing size and removed it completely for the most sterically hindered species (2′-*iso*propyl-, 2′,4′,6′-trimethyl-4NBp). In this case, the co-planarity of the nitro group is not affected but the twisting of the two aromatic rings, which is associated with a less effective charge delocalisation of the nitrenium ion.

The experimental mutagenicities of all nitro compounds were compared to predicted values, that are based on recently developed empirical equations. While there was reasonable correspondence for the parent compound (4NBp), its ortho methylated derivative (3-methyl-4NBp) and two highly hydrophobic dialkylated species (3,5-di*iso*propyl- and 3,5-di*tert*butyl-4NBp), predictions for all other alkyl substituted compounds were too high. Thus, steric parameters should be included to

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improve the general validity of predictions by means of quantitative structure–activity relationships (QSAR).

Keywords: *Salmonella*; Nitroaromatics; 4-Nitrobiphenyl; Alkyl substituents; Nitroso; Ortho; Mutagenicity; QSAR

1. Introduction

Aromatic and heteroaromatic nitro compounds are important sources for the production of dyes, polymers, pesticides, explosives (TNT), and pharmaceuticals (chloramphenicol). They are produced in large quantities industrially [1], but also as common intermediates in numerous research laboratories. Furthermore, these compounds are widespread environmental contaminants being found in diesel exhaust, combustion emissions, and airborne particulate matter in urban areas [2,3].

Besides the acute and chronic toxicity, nitroaromatics are generally mutagenic in bacterial test systems (e.g. *Salmonella typhimurium* reversion assay, Ames test) [2], and many of them are known for long to be carcinogenic [4]. Since their use involves risks to the human health, efforts are made to find less genotoxic substitutes of this type.

Today, it is generally accepted that the mutagenic properties of nitroaromatics result from the metabolic activation by mammalian and/or bacterial enzymes [5]. Reduction of the nitro group leads to nitroso compounds [6], hydroxylamines [7], and amines. Further activation of the hydroxylamines by O-acylation [8,9] produces electrophilic species (nitrenium ions) which react with bionucleophiles like proteins (e.g. hemoglobin) or DNA bases to form covalent adducts. Since these DNA adducts can disturb the replication process, mutations are induced. The overall induction rate appears to be dependent not only on the number and kind of adducts, but also on their persistence in vivo, and on the conformational heterogenicity [10] of the adduct modified DNA.

We are interested in general strategies to reduce the mutagenicity of genotoxic N-substituted aromatics like nitroaromatics. According to the relation between the mutagenic potential of nitroaromatics in *S. typhimurium* and their structural features, several aspects have already been investigated as, e.g. the position [11,12] and the orientation of the nitro substituent with respect to the ring plane [13–16], the ability of the corresponding aromatic system to stabilise the ultimate electrophiles, the molecular size and the planarity of the aromatic ring system, and the influence of additional substituents. All these features have a significant impact either on the adduct formation, persistence or conformational heterogenicity, and therefore on the mutagenicity.

Interestingly, little attention has been paid to the steric influence of alkyl substituents other than methyl on the mutagenicity of N-substituted aromatics. In aromatic amines [17] or their N-oxidised derivatives (hydroxylamines and nitroso compounds) [11] ortho methyl substituents often have an enhancing effect on the mutagenicity in the Ames test. In contrast, nitroaromatics like 4-nitrobiphenyl (4NBp) (**1**) bearing one or two additional ortho methyl groups are less mutagenic in comparison to the parent compound as shown by El-Bayoumy et al. [18] and Ashby et al. [19]. They proposed that this decrease of activity might be due to steric inhibition of the bacterial nitroreductase system. A similar reduction of mutagenicity was found when the methyl substituent was in the 2′-position of 4NBp **1** [18].

What is the effect of other alkyl substituents with increased steric demand like ethyl, *iso*propyl or *tert*-butyl groups? Is it possible to suppress mutagenicity of nitroaromatics completely? In this paper, we compared mutagenicities of alkyl substituted derivatives of 4NBp **1** (see Fig. 1). In the first two series consisting of the nitro compounds **2**–**8** and the nitroso compounds **1a** and **5a**, the influence of different ortho alkyl substituents was investigated, whereas in the third series, alkyl substituents in the 2′-position were varied (compounds **9**–**12**). All samples were tested for mutagenicity using the plate-incorporation assay (Ames test) with *S. typhimurium* tester strains TA98 and TA100. In all cases, samples were tested both with and without metabolic activation using rat liver S9 mix.

In recent years, several quantitative structure–activity relationship (QSAR) studies have been undertaken for an understanding of the structural parameters which determine mutagenicity. Among others Debnath and Hansch have developed empirical equa-

1	4-Nitrobiphenyl (92-92-3)	4NBp	-H
2	3-Methyl-4-nitrobiphenyl (69314-47-2)	3Me-4NBp	-Me
3	3-Ethyl-4-nitrobiphenyl (-)	3Et-4NBp	-Et
4	3-*iso*Propyl-4-nitrobiphenyl (127502-68-5)	3iPr-4NBp	-iPr
5	3-*tert*Butyl-4-nitrobiphenyl (70634-28-5)	3tBu-4NBp	-tBu
6	3,5-Diethyl-4-nitrobiphenyl (-)	3,5DiEt-4NBp	-Et
7	3,5-Di*iso*propyl-4-nitrobiphenyl (-)	3,5DiiPr-4NBp	-iPr
8	3,5-Di*tert*butyl-4-nitrobiphenyl (143726-80-1)	3,5DitBu-4NBp	-tBu
1a	4-Nitrosobiphenyl (10125-76-5)	4NOBp	-H
5a	3-*tert*Butyl-4-nitrosobiphenyl (-)	3tBu-4NOBp	-tBu
9	2'-Methyl-4-nitrobiphenyl (33350-73-1)	2'Me-4NBp	-Me
10	2'-Ethyl-4-nitrobiphenyl (-)	2'Et-4NBp	-Et
11	2'-*iso*Propyl-4-nitrobiphenyl (-)	2'iPr-4NBp	-iPr[a]
12	2', 4', 6'-Trimethyl-4-nitrobiphenyl (39117-68-5)	2',4',6'TMe-4NBp	

Fig. 1. Structure of synthesised compounds.

tions that allow to predict the mutagenicity of compounds in TA98 [20] and TA100 [21] (in the absence of S9 mix). In their equations, mutagenicity is mainly determined by hydrophobicity (log P values) and electronic energies (E_{LUMO}) — parameters which can be calculated nowadays by common methods. We have compared the predicted mutagenicities with the experimental data to establish if these equations are also applicable to the alkyl substituted compounds investigated in this work.

2. Materials and methods

2.1. Instrumentation

NMR spectra were obtained with Bruker spectrometers ARX-200 and AC-300 and are referenced to tetramethylsilane. Mass spectra were recorded with Variant MAT CH-7-A (EI, 70 eV). Elementary analyses were performed by a Heraeus Rapid Elementaranalysator.

2.2. Chemicals

4NBp **1** was obtained from Aldrich Chemical. 3-Alkyl- and 3,5-dialkyl-4NBps **2**–**8** were prepared according to a procedure described by Jendrella et al. [22]. Nucleophilic addition of appropriate alkyl Grignard reagents to 4NBp **1** at −78°C gave the Michael addition products which were rearomatised with 2,3-dichloro-5,6-dicyanobenzochinon (DDQ). Since the alkylation reaction was not regiospecific and the

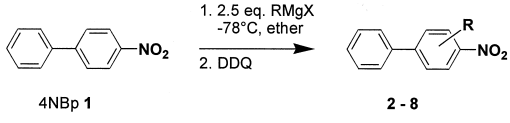
desired products had to be purified by repeated column chromatography, isolated yields were poor (about 10% for the monoalkylated species and about 5% for the dialkylated species) [23] (Scheme 1).

Compounds **9**–**12** were synthesised by palladium catalysed cross-coupling of alkyl substituted phenylboronic acids with 4-bromo-nitrobenzene (Aldrich Chemical) using the Suzuki protocol for sterically hindered compounds [24]. Yields vary from 73% to 92% [25] (Scheme 2).

Suzuki coupling of phenylboronic acid with 2-*tert*butyl-4-bromo-nitrobenzene, prepared by a reaction sequence of selective bromination [26] and oxidation [27] of commercially available 2-*tert*butylanilin (Aldrich Chemical) also produced compound **5** in 81% yield. The nitroso compounds **1a** and **5a** were prepared from the corresponding nitro compounds by reduction to the amines and mild reoxidation using the peroxy molybdenum complex $Mo(O)(O_2)_2$-(H_2O)(HMPA) [28]. All compounds were purified by recristallisation and/or repeated column chromatography to yield purities generally $>99\%$ except for 3iPr-4NBp **4** (a small amount of byproduct could not be separated completely, see below). Purities were checked by elemental analysis, gas chromatography and/or analytical HPLC. Analytical data of all synthesised compounds are given below.

*2.2.1. 3-Methyl-4NBp (**2**); typical procedure*

A solution of 6.57 g (33 mmol) 4NBp **1** in 120 ml THF was treated with 120 ml (80 mmol) methylmagnesiumiodide in diethyl ether (0.7 M) at -70°C and stirred for 1 h. After warming to -40°C, 7.56 g (33 mmol) DDQ was added, warmed to r.t., stirred for an additional hour and poured on water. The organic phase was evaporated in vacuo and the remaining aqueous phase was extracted with ethyl acetate ($3\times$ 300 ml). After washing with brine, the organic layer was dried ($MgSO_4$) and evaporated in vacuo. Repeated column chromatography (cyclohexane/methylene chloride 4:1) afforded **2** (0.64 g, 8.5%)



Scheme 1.

Scheme 2.

Orange crystals, mp 52–53°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 8.05$ (d, 1 H, $^3J = 9.0$ Hz, H5), 7.57–7.44 (m, 7 H, H_{arom}), 2.67 (s, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 147.0$, 146.0, 138.8, 134.3, 131.3, 129.0, 128.6, 127.3, 125.4 (2C), 20.9. MS (70 eV): m/z (%) = 213 (M^+, 100), 196 (M^+–OH ortho effect [29], 85). Calc. C 73.22%, H 5.19%, N 6.57%; found C 73.39%, H 5.48%, N 6.32%.

Analogously compounds **3**–**5** were prepared. The dialkylated species **6**–**8** were obtained as byproducts.

*2.2.2. 3-Ethyl-4NBp (**3**)*

Orange crystals, mp 54–55°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 8.00$ (d, 1 H, $^3J = 8.3$ Hz, H5), 7.60–7.45 (m, 7 H, H_{arom}), 3.01 (q, 2 H, $^3J = 7.5$ Hz $-CH_2-$), 1.33 (t, 3 H, $^3J = 7.5$ Hz $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 147.2$, 145.8, 139.5, 138.4, 129.6, 128.8, 128.3, 127.0, 125.1 (2C), 26.3, 14.7. MS (70 eV): m/z (%) = 227 (M^+, 70), 210 (M^+–OH ortho effect [29], 100). Calc. C 74.03%, H 5.72%, N 6.16%; found C 73.99%, H 5.74%, N 6.16%.

*2.2.3. 3-isoPropyl-4NBp (**4**)*

Deep red crystals, mp 44–46°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 7.79$ (d, 1 H, $^3J = 8.4$ Hz, H5), 7.64 (d, 1 H, $^4J = 1.9$ Hz, H2), 7.59 (dd, 2 H, $^3J = 8.0$ Hz, $^4J = 1.6$ Hz, H2′), 7.56–7.40 (m, 4 H, H_{arom}), 3.53 (sept, 1 H, $^3J = 6.8$ Hz $-CH$), 1.33 (d, 6 H, $^3J = 6.8$ Hz $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 148.6$, 145.6, 143.3, 139.4, 129.0, 128.5, 127.3, 126.2, 125.2, 124.6, 28.6, 23.6. MS (70 eV): m/z (%) = 241 (M^+, 29), 224 (M^+–OH ortho effect [29], 100). Calc. C 74.71%, H 6.22%, N 5.80%; found C 74.50%, H 5.71%, N 5.71% (95% by HPLC).

*2.2.4. 3-tertButyl-4NBp (**5**)*

Pale yellow crystals, mp 95°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 7.72$ (d, 1 H, $^4J = 1.8$ Hz, H5), 7.54 (dd, 2 H $^3J = 8.0$ Hz, $^4J = 1.6$ Hz, H2′), 7.40 (s, 1 H, H2), 7.46–7.38 (m, 4 H, H_{arom}), 1.45 (s, 9 H, $-C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 150.3$, 143.8, 141.9, 139.7, 129.0, 128.2, 127.5, 127.3, 125.4, 124.5, 35.8, 30.7. MS (70 eV): m/z (%) = 255 (M^+, 100), 238 (M^+–OH ortho effect [29], 18). Calc. C 75.31%, H 6.66%, N 5.48%; found C 75.50%, H 6.66%, N 5.48%.

*2.2.5. 3,5-Diethyl-4NBp (**6**)*

Light yellow crystals, mp 79–80°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 7.57$ (d, 2 H, $^3J = 8.0$ Hz, H2′), 7.48–7.38 (m, 3 H, H_{arom}), 7.34 (s, 2 H, H2), 2.64 (q, 4 H, $^3J = 7.5$ Hz $-CH_2-$), 1.28 (t, 6 H, $^3J = 7.5$ Hz–CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 143.5$, 139.8, 135.5 (2C), 128.8 (2C), 128.0, 127.2 (2C), 127.0, 125.9, (2C) 24.5, 14.9. MS (70 eV): m/z (%) = 255 (M^+, 100), 238 (M^+–OH ortho effect [29], 88). Calc. C 75.31%, H 6.66%, N 5.48%; found C 73.76%, H 6.71%, N 5.11%.

*2.2.6. 3,5-Diisopropyl-4NBp (**7**)*

Red crystals, mp 79–80°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 7.56$ (dd, 2 H, $^3J = 8.0$ Hz, $^4J = 1.6$ Hz, H2′), 7.54–7.40 (m, 3 H, H_{arom}), 7.36 (s, 2 H, H2), 2.88 (sept, 2 H, $^3J = 6.8$ Hz –CH–), 1.28 (d, 12 H, $^3J = 6.8$ Hz $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 149.6$, 143.4, 140.4, 139.6 (2C), 128.9 (2C), 128.0, 127.3 (2C), 123.1 (2C), 29.3, 23.8. MS (70 eV): m/z (%) = 283 (M^+, 50), 266 (M^+–OH ortho effect [29], 100), 43 (C_3H_7, 90). Calc. C 76.34%, H 7.41%, N 4.94%; found C 76.15%, H 7.55%, N 4.76%.

*2.2.7. 3,5-Ditertbutyl-4NBp (**8**)*

Yellow crystals, mp 121–122°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 7.60$ (dd, 2 H, $^3J = 8.0$ Hz, $^4J = 1.4$ Hz, H2′), 7.47–7.29 (m, 5 H, H_{arom}), 1.11 (s, 9H, $-C(CH_3)_3$), 1.07 (s, 9 H, $-C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 151.9$, 151.6 (2C), 141.0, 137.1, 128.7 (2C), 127.2 (2C), 126.3, 125.8, 78.2, 59.7, 28.2, 26.9 (broken symmetry). MS (70 eV): m/z (%) = 311 (M^+, 68), 296 (M^+–CH_3, 100). Calc. C 77.17%, H 8.04%, N 4.50%; found C 77.17%, H 8.12%, N 4.50%.

*2.2.8. 2′Methyl-4NBp (**9**); typical procedure*

Under argon, a mixture of 3.00 g (22 mmol) 2-methylphenylboronic acid, 4.04 g (20 mmol) 4-nitrobromobenzene, 9.46 g $Ba(OH)_2 * 8\ H_2O$ (30 mmol) and 120 mg $Pd(PPh_3)_4$ (0.22 mmol) in 100 ml dimethoxyethane and 20 ml degassed water was refluxed until 4-nitrobromobenzene had disappeared completely according to GC (10 h). After cooling, the mixture was poured onto water and extracted with ether (3×150 ml). The organic layer was separated, washed with brine, dried ($MgSO_4$), and evaporated to dryness in vacuo. Column chromatography (petrol ether/ethyl acetate 8:1) and recrystallisation (hexane) afforded **9** (3.30 g, 78%).

Pale yellow needles, mp 102–104°C (lit. 103°C [30]). ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 8.27$ (d, 2 H, $^3J = 8.8$ Hz, H3), 7.49 (d, 2 H, $^3J = 7.0$ Hz, H2), 7.31–7.20 (m, 4 H, H_{arom}), 2.27 (s, 3 H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 148.9$, 147.0, 139.7, 135.1, 130.8, 130.2 (2C), 129.5, 128.5, 126.2, 123.5 (2C), 20.4. MS (70 eV): m/z (%) = 213 (M^+, 77), 165 (100), 152 (M^+–CH_3–NO_2, 55). Calc. C 74.03%, H 5.72%, N 6.16%; found C 73.99%, H 5.74%, N 6.16%.

Analogously compounds **10**–**12** and **5** were prepared.

*2.2.9. 2′-Ethyl-4NBp (**10**)*

Colourless crystals, mp 80–81°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 8.27$ (d, 2 H, $^3J = 6.7$ Hz, H3), 7.48 (d, 2 H, $^3J = 6.7$ Hz, H2), 7.36–7.20 (m, 4 H, H_{arom}), 2.58 (q, 2 H, $^3J = 7.5$ Hz $-CH_2-$), 1.10 (t, 3 H, $^3J = 7.5$ Hz $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 148.9$, 147.0, 141.3, 139.1, 130.1, 129.5 (2C), 129.0, 128.7, 125.9, 123.4 (2C), 26.1, 15.6. MS (70 eV): m/z (%) = 227 (M^+, 65), 212 (M^+–CH_3, 13), 166 (M^+–NO_2–CH_3, 100), 165 (90). Calc. C 73.99%, H 5.77%, N 6.16%; found C 73.73%, H 5.97%, N 6.06%.

*2.2.10. 2′-isoPropyl-4NBp (**11**)*

Colourless crystals, mp 104–105°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 8.28$ (d, 2 H, $^3J = 8.7$ Hz, H3), 7.46 (d, 2 H, $^3J = 8.7$ Hz, H2), 7.40–7.44 (m, 2 H, H_{arom}), 7.22–7.28 (m, 1 H, H_{arom}), 7.15 (d, 2 H, $^3J = 7.2$ Hz, H_{arom}), 2.94 (sept, 1 H, $^3J = 6.9$ Hz –CH–), 1.17 (d, 6 H, $^3J = 6.9$ Hz $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 149.1$, 146.9, 146.1, 138.7, 130.2 (2C), 129.4, 128.8, 125.9, 125.7, 123.3 (2C),

29.6, 24.2. MS (70 eV): m/z (%) = 241 (M^+, 75), 226 (M^+–CH_3, 43), 180 (M^+–NO_2–CH_3, 100). Calc. C 74.67%, H 6.27%, N 5.80%; found C 74.44%, H 6.42%, N 5.80%.

Table 1
Mutagenicity of nitrobiphenyls with ortho alkyl groups

Code	Compound	Dose (μg/plate)	Revertants			
			TA98		TA100	
			− S9	+ S9	− S9	+ S9
	DMSO		30	45	116	117
	Positive control[a]		830	998	864	1033
1	4NBp	20	132	113	327	461
		100	464[b]	358	670[b]	1327
		500	0 tox	0 tox	0 tox	0 tox
		2500	0 tox	0 tox	0 tox	0 tox
		5000[c]	0 tox	0 tox	0 tox	0 tox
2	3Me-4NBp	20	83	206	518	373
		100	250[b]	363	1314[b]	962
		500	284	246	360	373
		2500	0 tox	0 tox	0 tox	0 tox
		5000[c]	0 tox	0 tox	0 tox	0 tox
3	3Et-4NBp	20	39	52	281	251
		100	55[b]	76	732[b]	630
		500	66	86	659 tox	783
		2500[c]	65	113	394 tox	465 tox
		5000[c]	69	126	335 tox	746 tox
4	3iPr-4NBp	20	34	75	276	325
		100	47[b]	173	327[b]	578
		500	81	186	597	903
		2500[c]	82	250	726	1035
		5000[c]	142	434	724	1626
5	3tBu-4NBp	20	31	44	117	131
		100	33	43	124	129
		500	39[b]	43	139[b]	188
		2500[c]	46	38	201	265
		5000[c]	46	27 tox	247	291
6	3,5DiEt-4NBp	20	32	46	122	157
		100	54	72	184	198
		500	91b	195	647[b]	418
		2500[c]	273	565	1270	741
		5000[c]	186 tox	423 tox	1297	1106
7	3,5DiiPr-4NBp	20	24	41	117	133
		100	31	56	111	168
		500	46[b]	149	119[b]	347
		2500[c]	45	292	130	699
		5000[c]	36	129 tox	123	730
8	3,5DitBu-4NBp	20	28	41	105	147
		100	28	33	106	140
		500	28	29	122[b]	128
		2500[c]	18	34	125	147
		5000[c]	14 tox	41	96 tox	141

[a] TA98-S9 NOPD (4-nitro-*o*-phenylenediamine) 10 μg; TA100-S9 MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) 5 μg; TA98 and TA100 + S9 2-aminoantracene 2.5 μg. Positive controls in Table 1 differ from those in Tables 2 and 3. Those in Table 1 were performed in a different laboratory than those in Tables 2 and 3.

[b] Indicates highest dose used in regression analysis.

[c] Precipitation: effective dose is lower than specified.

*2.2.11. 2′,4′,6′-Trimethyl-4NBp (**12**)*

Colourless crystals, mp 91°C. ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 8.30$ (d, 2 H, $^{3}J = 8.7$ Hz, H3), 7.35 (d, 2 H, $^{3}J = 8.7$ Hz, H2), 6.98 (s, br, 2 H, H_{arom}), 2.36 (s, 3 H, $-CH_3$), 2.00 (s, 6 H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) $\delta = 148.6$, 146.7, 137.7, 136.8, 135.2 (2C), 130.5 (2C), 128.4 (2C), 123.7 (2C), 21.0, 20.6 (2C). MS (70 eV): m/z (%) = 241 (M^+, 100), 165 ($M^+ - 2CH_3 - NO_2$, 78). Calc. C 74.67%, H 6.27%, N 5.80%; found C 74.43%, H 6.13%, N 5.89%.

*2.2.12. 3′-tertButyl-4-aminobiphenyl (**5b**)*

3′-*tert*Butyl-4NBp **5** (0.9 g, 3.52 mmol) and $SnCl_2$ (2.84 g, 15.0 mmol) were refluxed in 30 ml ethanol and 15 ml concentrated hydrochloric acid. The mixture was poured into 5 N NaOH and extracted with dichlormethane. The solution was dried ($MgSO_4$) and evaporated in vacuo. Column chromatography (petrol ether/diethyl ether 1:1) afforded **5b** (0.84 g, 89%) as in oil.

^{1}H-NMR ($CDCl_3$, 200 MHz) $\delta = 7.44$–7.14 (m, 7 H, H_{arom}), 6.57 (d, 1H, $^{3}J = 8.2$ Hz, H5), 3.69 (s, 2 H, $-NH_2$), 1.34 (s, 9 H, tBu). ^{13}C–NMR ($CDCl_3$, 50 MHz) $\delta = 144.0$, 141.7, 133.8, 131.4, 128.6, 128.2, 126.5, 126.1, 125.6, 118.1, 34.3, 29.6.

*2.2.13. 3-tertButyl-4-nitrosobiphenyl (**5a**)*

Molybenum(VI) oxide (3.0 g, 21 mmol) was dissolved in 30% H_2O_2 (100 ml) by gentle warming. After addition of hexamethyl phosphoric acid triamide (HMPA, 3.7 ml, 21 mmol) the mixture was stirred for 12 h. 3-*tert*Butyl-4-aminobiphenyl **5b** was dissolved in dichlormethane (20 ml) and the prepared $Mo(O_2)_2 * HMPT * H_2O$ solution (5 ml) was added. The mixture was stirred until the reaction had finished according to TLC (1 day), washed with water and brine, and evaporated in vacuo. The residue was purified by column chromatography (petrol ether/diethyl ether 1:1) and recrystallized from ethanol to yield **5a** (0.322 g, 61%).

Green crystals, mp 87°C. ^{1}H NMR ($CDCl_3$, 200 MHz) $\delta = 7.88$ (s, 1 H, H5), 7.60–7.31 (m, 6 H, H_{arom}), 6.08 (d, 1 H, $^{3}J = 8.4$ Hz, H2), 1.82 (s, 9 H, tBu). ^{13}C NMR ($CDCl_3$, 50 MHz) $\delta = 164.6$, 152.9, 147.7, 139.9, 129.0, 128.6, 127.4, 126.5, 124.5, 107.4, 37.1, 33.4. MS (70 eV): m/z (%) = 239 (M^+, 47), 222 (100), 210 (52), 207 (44), 196 (27), 178 (31), 167 (94), 152 (47), 77 (19). Calc. C 80.30% H 7.16% N 5.85%; found C 80.01% H 7.32%, N 5.65%.

Analogously compound **1a** was prepared.

2.3. Computational methods

Starting geometries for all compounds were obtained by MM2 force field calculations from structures constructed from standard bond lengths and bond angles. The initial conformation of the nitro group was planar with respect to the aromatic ring, the initial dihedral angle between the phenyl rings was chosen for all compounds to be 45°, and dihedral angles for ethyl and isopropyl groups and the aromatic ring were varied from 0° to 120° in 30° steps. Full geometry optimisation was achieved using the semiempirical AM1 method developed by Dewar et al. [31]. Structural parameters as dihedral angles were taken from structures after full geometry optimisation. LUMO energies E_{LUMO} were calculated from the conformer with the lowest heat of formation. All calculations were carried out with the program MOPAC V6.0 using the key words EF GNORM = 0.040 GEO-OK AM1 XYZ PRECISE LET DDMIN = 0.0. The log P coefficients for all compounds were calculated by means of the program

Table 2
Mutagenicity of nitrosobiphenyls **1a** and **5a**

Code	Compound	Dose (μg/plate)	Revertants			
			TA98		TA100	
			−S9	+S9	−S9	+S9
	DMSO		32	43	158	146
	Positive control[a]		3136	1628	892	2351
1a	4NOBp	10	249	340	557[b]	769
		25	516[b]	778	311	1112
		50	513	1111	272	1005
		100	347	1104	167	531
		500	0	614	0	51
5a	3tBu-4NOBp	10	119	85	855	418
		25	271[b]	232	1386[b]	963
		50	405	463	1620	1626
		100	596	713	1386	2178
		500	874	1032	855	1810

[a] TA98-S9 nitropyrene 2.5 μg; TA100-S9 sodiumazide 5 μg; TA98 and T100 + S9 2-aminoantracene 2.5 μg.

[b] Indicates highest dose used in regression analysis.

KOWWIN V1.54. Calculated octanol/water partition coefficients usually show good agreement with the experimental log *P* values.

2.4. Mutagenicity tests

The Ames test protocol employed in these investigations strictly followed the standard methodology described by Maron and Ames [32]. Both tester strains (TA98 and TA100) were a gift from Bruce Ames, arochlor-induced S9 from rat liver was purchased from CCR, Germany.

It should be noted that variations in the methodology employed in other laboratories and/or variations in the quality of the agar used or the protein content and activity of the S9, can all influence the results of Ames tests. Consequently, the comparability with published data can be poor and conclusions drawn from the comparison of data sets from different laboratories should be viewed with caution, particularly in cases where the exact details of the methodology used are not given.

In this study, sample dilutions were prepared for each test compound (**1**–**12**) from the maximum indicated in Tables 1–3. In all cases, DMSO was used as the diluting solvent. Positive and negative controls (DMSO) were included in each experiment. Each sample was examined in triplicate and all experiments were performed twice on random separate dates. The test results shown here were obtained

Table 3
Mutagenicity of nitrosobiphenyls with alkyl groups in the 2′-position

Code	Compound	Dose (μg/plate)	Revertants			
			TA98		TA100	
			− S9	+ S9	− S9	+ S9
	DMSO		37	67	191	206
	Positive control[a]		2721	1151	902	795
1	4NBp	20	96	131	347	488
		100	233[b]	406	795[b]	1285
		500	290	508	885	1294
		2500	382	660	904	1377
		5000[c]	721	830	1177	1567
9	2′Me-4NBp	20	55	57	191	204
		100	100	79	237	244
		500	382[b]	348	339[b]	393
		2500	240 tox	173 tox	347	579
		5000[c]	58 tox	161 tox	349	426 tox
10	2′Et-4NBp	20	54	56	185	226
		100	53[b]	57	189	232
		500	49	57	200[b]	234
		2500	48	60	219	258
		5000[c]	67	65	302	349
11	2′iPr-4NBp	20	49	58	177	189
		100	55[b]	40	170	199
		500	51	43	196[b]	199
		2500	51	44	212	205
		5000[c]	35 tox	45	271	208
12	2′,4′,6′TMe-4NBp	20	45	49	189	203
		100	49[b]	57	184	204
		500	58	53	183	200
		2500	59	48	219[b]	217
		5000[c]	44	50	274	269

[a] TA98-S9 nitropyrene 2.5 μg; TA100-S9 sodiumazide 5 μg; TA98 and TA100 + S9 2-aminoantracene 2 μg.
[b] Indicates highest dose used in regression analysis.
[c] Precipitation: effective dose is lower than specified.

using metabolic activation with S9 mix (+S9) and without metabolic activation (−S9). In each case, a five-point dose–response curve was established using appropriate dilutions of the compound to be tested. To detect even very weak genotoxic and cytotoxic effects, all compounds were tested in a wide dosage range (up to 5000 μg). To guarantee optimal comparability all compounds within one series were tested under identical conditions within 1 week.

3. Results

The number of revertants of 4NBp **1**, its alkylated derivatives **2**–**12** and the nitroso compounds **1a** and **5a** are shown in Tables 1–3.

3.1. Compounds containing alkyl groups ortho to the nitro / nitroso group (Tables 1 and 2)

As expected, the parent compound 4NBp **1** induced in both *Salmonella* strains with and without metabolic activation high revertant numbers. Striking is the high cytotoxocity of **1** killing all bacteria at concentrations above 500 μg. Most of our data for 3Me-4NBp **2** are in good agreement with the results reported by El-Bayoumy et al. [18]: 3Me-4NBp **2** exhibited a similar mutagenicity like 4NBp **1** but was less cytotoxic. When assayed in TA98 without S9 a 46% mutagenicity reduction compared to 4NBp **1** was observed, while revertant numbers were similar to 4NBp **1** in TA98 with S9, and in TA100 with S9. We found a significant increase of activity only in TA100 without S9, while El-Bayoumy observed in this case a slight reduction of mutagenicity. Compared to 3Me-4NBp **2**, the ethyl substituted compound 3Et-4NBp **3** was less mutagenic in both strains. Cell toxicity was observed in TA100 when assayed at doses above 100 μg. While 3iPr-4NBp **4** was slightly less active in TA100 and TA98 without S9 mix, unexpectedly we found an increased mutagenicity in TA98 with metabolic activation. However — as mentioned above — this compound contained an impurity, which could not be characterised. The increased mutagenicity in TA98 + S9 may be due to this impurity. Finally 3tBu-4NBp **5** with its bulky tertiary butyl group was the weakest mutagen in TA100 in this series, and was non-mutagenic in TA98.

Compared to the monoalkylated compounds **3**–**5**, the mutagenicity of the corresponding dialkylated compounds **6**–**8** was generally lower. Regarding the steric bulk of the dialkylated species **6**–**8**, a similar trend as above was observed. The diethyl compound 3,5DiEt-4NBp **6** was significantly less mutagenic and less cytotoxic than the parent compound **1** but produced still high revertant numbers at doses over 500 μg. The sterically even more hindered diisopropyl compound 3,5DiiPr-4NBp **7** exhibited activity at this high dosages only in the presence of S9, while it was non-mutagenic in the absence of S9. 3,5DitBu-4NBp **8** with its two tertiary butyl groups was non-mutagenic.

In both test strains, the unsubstituted nitroso compound **1a** (Table 2) was much more mutagenic than the corresponding nitro compound **1** underlining the fact that the initial nitro reduction is an important activation process. When assayed in TA100 without S9 mix **1a** exhibited high cytotoxicity at concentrations above 10 μg and revertant numbers at higher concentrations remained low. Remarkably, in TA98 the ortho *tert*butyl group of 3tBu-4NOBp, **5a** decreased the mutagenic potential only weakly and — amazingly — in TA100 this group even enhanced the mutagenicity if compared to **1a**.

3.2. Compounds containing alkyl groups in the 2′-position (Table 3)

In the third test series, the compounds **9**–**12**, containing different alkyl substituents in the 2′-position of the biphenyl system, and — for reasons of comparison — 4NBp **1** were investigated. Interestingly, the cytotoxicity of the parent compound 4NBp **1** turned out to be much lower than in the first series. Since the third series was performed in a different lab (see footnote in Table 1) using the same test protocol and the same 4NBp **1**, these deviations underline the necessity to carry out Ames tests in the same lab if quantitative results should be compared.

Most significantly as in the first series, a strong influence of the alkyl groups on the mutagenicity was observed too. Apart from 4NBp **1,** only the methyl substituted compound 2′Me-4NBp **9** exhibited moderate activity in both test strains, in good

agreement with the results of El-Bayoumy et al. [18]. The mutagenicity of 2′Et-4NBp **10**, however, was weak even at the highest doses, and compounds **11** and **12** showed no activity.

Thus, introduction of sterically demanding alkyl substituents either ortho to the nitro group or in the 2′-position of 4NBp reduced the mutagenicity dramatically or even eliminated it completely. This effect was not observed for the corresponding nitroso compounds!

4. Discussion

4.1. Comparison of predicted and experimental mutagenicities

The mutagenic activity of nitroaromatics in *Salmonella* strains TA98 and TA100 (in the absence of S9) is mainly determined by hydrophobicity and electronic factors. This conclusion was drawn in recent QSAR investigations by Debnath et al. [20,21]. Based on the analyses of almost 200 nitroaromatics, their investigations led to the following two empirical Eqs. 1 and 2 (indicator variables omitted):

$$\log \mathrm{TA98} = 0.65 \log P - 2.90 \log(\beta 10^{\log P} + 1) - 1.38 E_{\mathrm{LUMO}} - 4.15 \qquad (\log \beta = -5.48) \tag{1}$$

$$\log \mathrm{TA100} = 1.20 \log P - 3.40 \log(\beta 10^{\log P} + 1) - 2.05 E_{\mathrm{LUMO}} - 6.39 \qquad (\log \beta = -5.70) \tag{2}$$

These equations describe a bilinear correlation between the mutagenicity of nitro compounds and their hydrophobicity ($\log P$). Mutagenicity increases with hydrophobicity until an optimal $\log P$ is reached ($\log P_{\max} \approx 5$) and then drops rapidly for more hydrophobic compounds. According to the electronic factors, a linear dependence between mutagenicity (TA98 and TA100) and E_{LUMO} was found, that has been attributed to the initial reduction of the nitro group as the rate-limiting step in nitroarene activation [20]. It means that activity in both strains is enhanced if the compound is more easily reduced. Despite the large range of structures used to develop these equations, essentially variations of the aromatic ring system were covered. Substituent effects on the other hand have not been carefully studied. Being aware of this restriction, the authors stated that ''it is important to investigate the hydrophobic effect of aliphatic side chains on the mutagenicity to see if it parallels that of the flat aromatic systems'' [20].

In Table 4, the $\log P$ values, the LUMO energies, the predicted and the experimental mutagenicities of the alkyl substituted nitrobiphenyls (**1**–**12**) are summarised. The number of revertants induced by the test compound (rev/nmol) was calculated using regression analysis over the linear portion of the dose–response curve (usually including 0, 20, 100 μg). The $\log P$ values increase from 4NBp **1** with the number and size of the alkyl substituents to reach values over 6 for the dialkylated compounds 3,5DiiPr-4NBp **7** and 3,5DiTBu-4NBp **8**. Although prediction of high $\log P$ values (> 6) often is not completely accurate, the general trend is correct. The LUMO energies are raised almost in the same order because of the +I effect of the alkyl substituents. A graphical comparison of predicted and experimental mutagenicities is given for all compounds in Figs. 2–4. Logarithmical mutation rates below −2 (rev/nmol < 0.01) were generally set to −2.

With regard to compounds **1**–**5** (Fig. 2) the activity in both strains TA98 and TA100 is predicted to increase from the parent compound 4NBp **1** via 3Me-4NBp **2** to the ethyl substituted species 3Et-4NBp **3** and then — while passing the optimal $\log P$ value — to decrease from 3iPr-4NBp **4** to 3tBu-4NBp **5**. The experimental trends are somewhat different. In TA98, we observed a continuous decrease of mutagenicity from 4NBp **1** to 3tBu-4NBp **5**. In TA100, after an initial increase for 3Me-4NBp **2**, a similar activity reduction via **3** and **4** to 3tBu-4NBp **5** was detected. Thus, there is good agreement between predicted and experimental results for the parent compound **1** and its methylated derivative **2**, but deviations become larger with increasing size of the alkyl groups (**3** ⇒ **4** ⇒ **5**). These deviations point to an effect which is not explicitly incorporated in Eqs. 1 and 2.

Table 4
Structural parameters, predicted and experimental mutagenicity

Code	Compound	log P[a]	E_{LUMO}	log Revertants/nmol				Dihedral angles[b]	
				TA98		TA100		$C^{2'}C^{1'}C^{1}C^{2}$	$C^{3}C^{4}NO$
				pred.	exp.	pred.	exp.	ring twisting	NO_2 orientation
1	4NBp	3.57	−1.229	−0.15	−0.07	0.40	0.02	40.3	0.0
2	3Me-4NBp	4.12	−1.175	0.10	−0.34	0.92	0.38	40.5	0.0
3	3Et-4NBp	4.61	−1.159	0.29	−1.27	1.40	0.14	40.8	0.0
4	3iPr-4NBp	5.03	−1.089	0.24	−1.39	1.59	−0.39	41.3	28.8
5	3tBu-4NBp	5.48	−0.812	−0.34	< −2	1.15	−1.68	42.2	75.8
6	3,5DiEt-4NBp	5.65	−0.959	−0.30	−1.51	1.42	−0.56	41.8	44.1
7	3,5DiiPr-4NBp	6.49	−0.781	−1.90	−1.96	0.09	< −2	41.6	69.9
8	3,5DitBu-4NBp	7.39	−0.688	< −2	< −2	−1.89	< −2	43.2	85.6
1a	4NOBp	3.63	−0.979	–	0.55	–	0.86	40.2	0.0
5a	3tBu-4NOBp	5.54	−0.907	–	0.36	–	1.06	40.9	3.1
1	4NBp	3.57	−1.229	−0.15	−0.42	0.40	0.07	40.3	0.0
9	2′Me-4NBp	4.12	−1.168	0.09	−0.72	0.91	−1.09	51.2	0.0
10	2′Et-4NBp	4.61	−1.108	0.22	−1.60	1.30	< −2	65.4	0.0
11	2′iPr-4NBp	5.03	−1.126	0.29	< −1.5[c]	1.66	< −2	59.5	0.0
12	2′,4′,6′-TMe-4NBp	5.21	−1.050	0.14	< −1.5[c]	1.60	< −2	78.0	0.0

[a] Log P values calculated with KOWWIN slightly differ from those calculated with CLOGP which was used by Debnath and Hansch.
[b] Geometries were fully optimised using AM1 method.
[c] No linear dose response found.

In contrast, with regard to the even more hydrophobic dialkylated species **6**–**8** the decreasing activity in both strains is well predicted (Fig. 3). In TA100, the prediction is less precise than for TA98 but the overall trend is still accurate. Debnath and Hansch proposed that in nitroaromatics (without alkyl substituents), the activity drop for more hydrophobic compounds (log > 6) may be due to a combination of adverse hydrophobic and steric effects [21]. Our results suggest that high hydrophobicity generally leads to non-mutagenic compounds independent whether the hydrophobicity is due to a pure aromatic or to an alkyl substituted skeleton.

For compounds **1**,**9**–**12** (Fig. 4), similar curves are observed, as for compounds **1**–**5**. While an increasing mutagenicity is predicted, a continuous decrease was found. In analogy to Fig. 2, deviations grow with increasing steric demand, indicating that alkyl groups account for effects that cannot be modeled suitably by the hydrophobicity and E_{LUMO} of the corresponding alkyl substituted nitro compound alone.

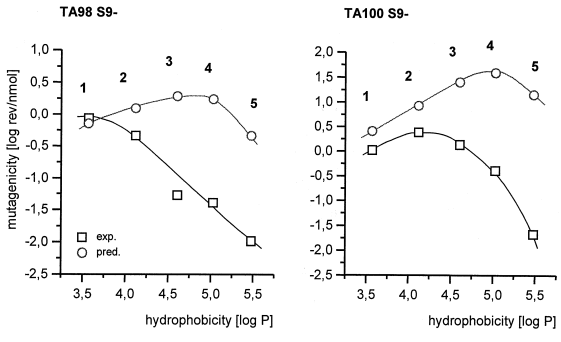


Fig. 2. Experimental and predicted mutagenicities of compounds **1**–**5**.

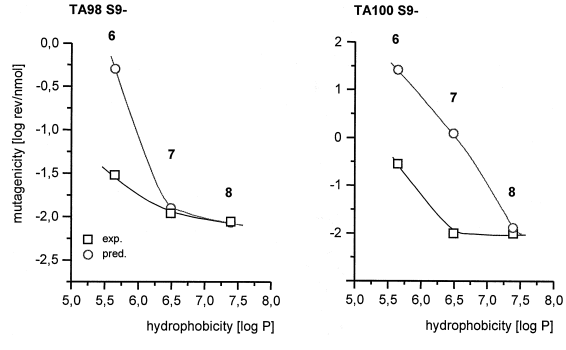


Fig. 3. Experimental and Predicted mutagenicities of compounds **6**–**8**.

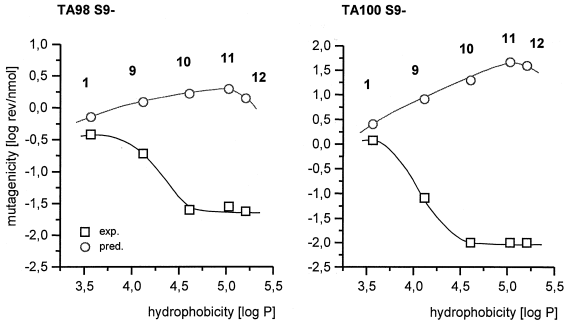


Fig. 4. Experimental and Predicted mutagenicities of compounds **1**,**9**–**12**.

4.2. Reduction of mutagenicity — possible explanations

To gain further understanding in the mode of inhibition by bulky ortho alkyl groups, we had a closer look to the orientations of the nitro groups. Fu et al. [14,15] and Jung et al. [16] reported recently that (non substituted) polycyclic nitroaromatics exhibit either only weak or no direct mutagenic activity if the nitro group is oriented perpendicular or nearly perpendicular to the aromatic plane. This hypothesis is also true for the ortho alkyl substituted nitroaromatics of this work. Indeed compounds **1**–**8** show a good correlation between the orientations of the nitro groups and the experimental mutagenicities (Table 4). While in the parent compound **1** and its methyl (**2**) and ethyl (**3**) derivatives the nitro groups are orientated coplanar to the ring and therefore fully conjugated with the aromatic system, in 3iPr-4NBp **4** and 3,5DiEt-4NBp **6**, both weak mutagens, the nitro groups are found to be twisted significantly. Moreover in 3tBu-4NBp **5**, 3,5DiiPr-4NBp **7** and 3,5DitBu-4NBp **8**, which are essentially non-mutagenic, nearly perpendicular orientations of the nitro groups to the phenyl systems are calculated. Similar twistings are experimentally observed from crystal structures of ortho alkylated nitro compounds, as, e.g. of 1,5-di*tert*butyl-2,4-dinitrobenzene [33] and 1,3,5-triipropyl-2-nitrobenzene [34] with dihedral angles of 65° and 84°, respectively. These deviations from the electronically more favoured coplanar orientation are due to the steric repulsion between the nitro group and the neighbouring alkyl substituents.

Fu suggested that the decreased mutagenicity of polycyclic nitroaromatics with perpendicular nitro groups may be simply due to their inability to fit into the active site of the nitroreductase [14]. Indeed, the orientation of the nitro group seems to determine the inhibition of activity rather than the steric demand of the alkyl groups themselves. This is supported by our observation that 3-*tert*butyl-4-nitrosobiphenyl **5a**, a potential reduction intermediate of 3tBu-4NBp **5**, is a potent mutagen (**5a**: TA98-S9, log rev/nmol = +0.36; TA100-S9, log rev/nmol = +1.06) if compared to **5** (TA98-S9, log rev/nmol < −2; TA100-S9, log rev/nmol = −1.68). Although bearing the bulky *tert*butyl group, **5a** is hardly less mutagenic than the non-substituted 4-nitrosobiphenyl (**1a**) in TA98 and even more mutagenic in TA100 (see Table 4). In contrast to the different orientations of the nitro groups in **1** and **5**, the nitroso groups in compounds **5a** and **1a** are both orientated coplanar to the aromatic plane and the nitroso reduction is not inhibited. Nevertheless, for very hydrophobic compounds as **7** and **8** (log $P > 6$) low solubility may play a role for inhibition of mutagenicity as well.

According to the series of the 2′-alkylated compounds the decrease of mutagenicity (**1** > **9** > **10** ~ **11** ~ **12**) is also correlated with structural features as well. El-Bayoumy suggested for compound **9**, that the methyl group interferes with the extensive charge delocalization over both aromatic rings by causing a deviation of the biphenyl system from coplanarity [18]. The dihedral angles between the phenyl groups are shown in Table 4. While in 4NBP **1** and all ortho-alkylated compounds the calculated dihedral angles lie within a range of 40–43°, alkyl substituents in the 2′-position cause significant deviations to larger angles changing the molecular shape from weakly to strongly twisted. This is especially true for 2′Et-4NBp **10**, 2′iPt-4NBp **11**, and 2′,4′,6′TMe-4NBp **12**, which are all non-mutagenic. In fact, these compounds with nearly orthogonal phenyl rings approach the properties of nitrobenzenes. Chiu et al. [35] has shown that nitrobenzene is non-mutagenic in *S. typhimurium*. Probably the weaker π-overlapping destabilises the intermediate biphenyl nitrenium ions and reduces their lifetime. Alternatively, the three-dimensional structure may inhibit the approach to the active side of the reduction enzymes.

In conclusion, it is shown, that non-mutagenic derivatives of 4NBp **1** are obtained by introduction of bulky alkyl substituents ortho to the nitro group or in 2′-position. For most of such alkyl substituted compounds, mutagenicities are predicted by QSAR as being too high since the existing equations do not take into account steric effects of aliphatic substituents. These results should be transferable to other nitroaromatics with a similar substitution pattern as well as, e.g. ortho alkylated nitrofluorenes and nitropyrenes. The influence of alkyl groups at other positions that neither affect the orientation of the nitro group nor the charge delocalization will be shown in the following paper [36]. The main effect discussed in that following paper — namely the reduction of an efficient intercalation into DNA for steric reasons — may also play a role for the decreased activity observed here. The mutagenic properties of alkylated aromatic amines will be discussed elsewhere.

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References

[1] D.R. Hartter, The use and importance of nitroaromatic chemicals in the chemical industry, in: D.E. Rickert (Ed.), Toxicity of Nitroaromatic Compounds, Hemisphere, New York, 1985, pp. 1–13.

[2] H. Tokiwa, Y. Ohnishi, Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment, CRC Crit. Rev. Toxicol. 17 (1986) 23–60.

[3] H. Tokiwa, N. Sera, M. Kai, K. Horikawa, Y. Ohnishi, The role of nitroarenes in the mutagenicity of airborne particles indoors and outdoors, in: M.D. Waters (Ed.), Genetic Toxicology of Complex Mixtures, Plenum, New York, 1990, pp. 165–172.

[4] H.P. Morris, C.S. Dubnik, J.M. Johnson, Studies of the carcinogenic action in the rat of 2-nitro, 2-amino-, 2-acetylamino-, and 2-diacetylaminofluorene after ingestion and after painting, J. Natl. Cancer Inst. 10 (1950) 1201–1212.

[5] F.A. Beland, F.F. Kadlubar, Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons, in: C.S. Cooper, P.L. Grover (Eds.), Chemical Carcinogenesis and Mutagenesis, Handbook exp. Pharmacol. I/8, 267–325.

[6] R.H. Heflich, P.C. Howard, F.A. Beland, 1-Nitrosopyrene: an intermediate in the metabolic activation of 1-nitropyrene in *Salmonella typhimurium* TA1538, Mutat. Res. 149 (1985) 25–32.

[7] S. Ning, X. Xiaobai, Reductive metabolism of 4-nitrobiphenyl by rat liver fraction, Carcinogenesis 18 (6) (1997) 1233–1240.

[8] K. Saito, Y. Yamazoe, T. Kamataki, R. Kato, Mechanism of activation of proximate mutagens in ames tester strains: the acetyl-CoA dependent enzyme in *Salmonella typhimurium* TA98 deficient in TA98/1,8-DNP6 catalyses DNA-binding as the cause of mutagenicity, Biochem. Biophys. Res. Commun. 116 (1983) 141–147.

[9] N. Sera, K. Kukuhara, N. Miyata, H. Tokiwa, Mutagenicity of nitrophenanthrene derivatives for *Salmonella typhimurium*: effects of nitroreductase and acetyltransferase, Mutat. Res. 349 (1996) 137–144.

[10] L. Zhou, M. Rajabzadeh, D. Traficante, B.P. Cho, Conformational heterogeneity of arylamine-modified DNA: 19F evidence, J. Am. Chem. Soc. 119 (1997) 5384–5389.

[11] S.S. Hecht, K. El-Bayoumy, L. Tulley, E. LaVoie, Structure-mutagenicity relationships of N-oxidised derivatives of aniline, o-toluidine, 2′-methyl-4-aminobiphenyl, and 3,2′-dimethyl-4-aminobiphenyl, J. Med. Chem. 22 (1979) 981–987.

[12] M. Nohara, T. Hirayama, Y. Fujioka, S. Ozasa, E. Ibucki, S. Fukui, Relationship between mutagenic potency in *Salmonella typhimurium* and chemical structure of amino- and nitro-substituted biphenyls, Mutat. Res. 149 (1985) 9–15.

[13] W.A. Vance, D.E. Levin, Structural features of nitroaromatics that determine mutagenic activity in *Salmonella typhimurium*, Environ. Mutagen. 6 (1984) 797–811.

[14] P.P. Fu, M.W. Chou, D.W. Miller, G.L. White, R.H. Heflich, F.A. Beland, The orientation of the nitro-substituent predicts the direct-acting bacterial mutagenicity of nitrated polycyclic aromatic hydrocarbons, Mutat. Res. 143 (1985) 173–181.

[15] P.P. Fu, M.W. Chou, F.A. Beland, Effects of nitro substitution on the in vitro metabolic activation of polycyclic aromatic hydrocarbons, in: S.K. Yang, B.D. Silverman (Eds.), Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure–Activity Relationships, CRC Press, Boca Raton, FL, 1988, pp. 37–65.

[16] H. Jung, A.U. Shaikh, R.H. Heflich, P.P. Fu, Nitro group orientation, reduction potential and direct-acting mutagenicity of nitro-polycyclic aromatic hydrocarbons, Environ. Mol. Mutagen. 17 (1991) 169–180.

[17] K. El-Bayoumy, E.J. LaVoie, L. Tulley-Freiler, S.S. Hecht, Effects of ortho-methyl substituents on the mutagenicity of aminobiphenyls and aminonaphthalenes, Mutat. Res. 90 (1981) 345–354.

[18] K. El-Bayoumy, E.J. Vavoie, S.S. Hecht, E.A. Fow, D. Hoffmann, The influence of methyl substitution on the mutagenicity of nitronaphthalenes and nitrobiphenyls, Mutat. Res. 81 (1981) 143–153.

[19] J. Ashby, D. Paton, P.A. Lefevre, J.A. Styles, F.L. Rose, Evaluation of two suggested methods of deactivating organic carcinogens by molecular modification, Carcinogenesis 3 (1982) 1283–1287.

[20] A.K. Debnath, R.L. Lopez de Compadre, G. Debnath, A.J. Shusterman, C. Hansch, Structure–activity relationship of mutagenic aromatic and hetreoaromatic nitro compounds. Correlation with molecular orbital energies and hydrophobicity, J. Med. Chem. 34 (1991) 786–797.

[21] A.K. Debnath, R.L. Lopez de Compadre, A.J. Shusterman, C. Hansch, Quantitative structure–activity relationship investigation of the role of hydrophobicity in regulating mutagenicity in the ames test: 2. Mutagenicity of aromatic and heteroaromatic nitro compounds in *Salmonella typhimurium* TA100, Environ. Mol. Mutagen. 19 (1992) 53–70.

[22] H. Jendrella, E. Granzer, B.v. Kerekjarto, Synthesis and biological activity of new HMG-CoA reductase inhibitors: 3. Lactones of phenoxy-3,5-dihydroxyhexanoic acids, J. Med. Chem. 34 (1991) 2962–2983.

[23] U. Voigtmann, Mutagenitätsuntersuchungen an ortho-alkylierten 4-nitrobiphenylen, Diplomarbeit, Philipps-Universität Marburg, 1996.

[24] A. Suzuki, T. Watanabe, N. Miyaura, Synthesis of sterically hindered biaryls via the palladium-catalysed cross-coupling reaction of arylboronic acids or their esters with haloarenes, Synlett (1992) 207–210.

[25] M. Klein, Mutagenitätsuntersuchungen an substituierten 4-nitrobiphenylen, Diplomarbeit, Philipps-Universität Marburg, 1997.

[26] V. Calo, L. Lopez, A selective bromination of aromatic amines, J. Chem. Soc. C (1971) 3652–3653.

[27] K. Krohn, J. Küpke, H. Rieger, Zirkonium katalysierte oxidation von primären aromatischen Aminen zu nitroverbindungen mit tert-butylhydroperoxid, J. Prakt. Chem. 339 (1997) 335–339.

[28] S. Tollari, M. Cuscela, F. Porta, Catalytic oxidation of primary aromatic amines to the corresponding nitroso compounds by H_2O_2 and $Mo(O)(O_2)_2(H_2O)$(HMPA), Chem. Commun. (1993) 1510–1511.

[29] M. Hesse, H. Meier, B. Zeeh, in: Spektrokopische Methoden in der Organischen Chemie, Georg. Thieme Verlag, Stuttgart, 1991, p. 248.

[30] H. France, I.M. Heilborn, D.H. Hey, Studies in the Terphenyl Series: Part II. Hydroxy- and Methyl-*p*-terphenyls, J. Chem. Soc. (1939) 1283–1287.

[31] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, AM1: a new general purpose quantum mechanical molecular model, J. Am. Chem. Soc. 107 (1985) 3902–3909.

[32] D.M. Maron, B.N. Ames, Revised methods for the Salmonella mutagenicity test, Mutat. Res. 113 (1983) 173–215.

[33] M. Numann, H. Schenk, D. Döpp, Structure of 1,3,5-triisopropyl-2-nitrobenzene, Acta Crystallogr., Sect. C 51 (1995) 260–266.

[34] J.A. DeRidder, H. Schenk, D. Döpp, 1,5-Di-tert-butyl-2,4-dinitrobenzene, Acta Crystallogr., Sect. C 49 (1993) 1970–1974.

[35] C.W. Chiu, L.H. Lee, C.Y. Wang, G.T. Bryan, Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*, Mutat. Res. 58 (1978) 11–22.

[36] M Klein, L. Erdinger, G. Boche, From mutagenic to non-mutagenic nitroarenes: effect of bulky alkyl substituents on the mutagenic activity of nitroaromatics in *Salmonella typhimurium*. Part II. Substituents far away from the nitro group, Mutat. Res. 467 (2000) 69–82, this issue.