Tetrahedron Letters, Vol.30, No.3, pp 321-324, 1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

Synthesis of N-Acetoxy-2-aminonaphthaline, an Ultimate Carcinogen of the Carcinogenic 2-Naphthylamine, and Its In Vitro Reactions with (Bio)Nucleophiles

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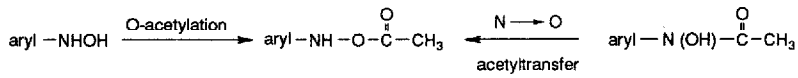
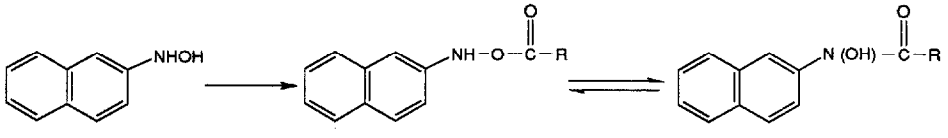
Summary: In this communication we describe (1) the synthesis of N-acetoxy-2-aminonaphthaline 4a (an ultimate carcinogen of the carcinogenic 2-naphthylamine), of N-pivaloyloxy-2-aminonaphthaline 4b, and (2) the reactions of 4a(b) with the nucleophiles N-methylaniline 6 and deoxyguanosine 7. Of special interest is the formation of the deoxyguanosine "adducts" 12-14.

Recent investigations[1j] indicate the N-acetoxy derivatives of carcinogenic arylamines **1** to be important ultimate carcinogens in the carcinogenesis of aromatic amines[1]. They are formed via enzymatic O-acetylation of the corresponding hydroxylamines **2**[2] and/or N -> O acetyl transfer from the corresponding hydroxamic acids **3**[3].



2 **1** **3**

In this communication we report first on the synthesis of N-acetoxy- and N-pivaloyloxy-2-aminonaphthaline **4a**[4] and **4b**, respectively, via O-acylation of the hydroxylamine **5**, secondly we describe the reactions of **4a(b)** with the model nucleophile N-methylaniline **6** and with deoxyguanosine **7**. Thirdly, we report on the base catalyzed transformations of the hydroxamic acids **8a** and **8b** into the corresponding O-acyl derivatives **4a** and **4b**, and their reactions with **6**.



5 **4** **8**

a : R = CH_3

b : R = $C(CH_3)_3$

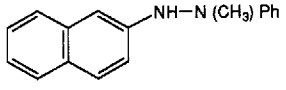
6 : N-Methylaniline

7 : Deoxyguanosine

4a(b) were prepared by reaction of **5** with acetylcyanide (pivaloylcyanide) and triethylamine in ether at low temperatures[5-7]. The thermally very instable

compounds were characterized by low temperature IR, ^{13}C and ^{1}H NMR spectroscopy[5].

Reactions of **4a(b)** with N-methylaniline **6** in **6** as the solvent at 20°C led to the following products[8]:



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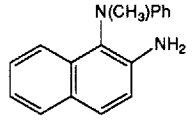

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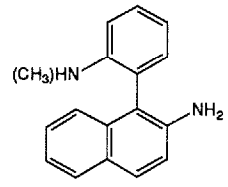


11

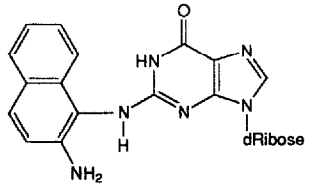
The yields of **9**, **10** and **11** are given in Tab. 1.

Tab.1. Yields of **9**, **10** and **11** in the reactions of **4a**, **4b**, **8a** and **8b**, respectively, with N-methylaniline **6**.

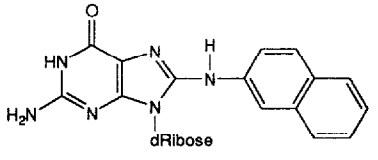
	reaction conditions	yields [%]		
		9	**10**	**11**
4a	in **6**, 20°C, 0.5 h	32	48	6
4b	in **6**, 20°C, 0.5 h	42	42	11
8a	in **6**, 80°C, 2 mol. equiv. DBU, 1.5 h	31	37	6
8b	in **6**, 50°C, 2 mol. equiv. DBU, 0.5 h	40	48	-

Hydrazines like **9** have also been formed in reactions of N-acyloxyphenylamines with amines[7]. Ring amination products like **10** and **11** so far have only been detected in the reactions of N-acyloxy-4-aminobiphenyl with amines[9]. **9**, **10** and **11** are also formed if the hydroxamic acids **8a(b)** are reacted with N-methylaniline **6** in the presence of DBU (see Tab. 1)[8] . This indicates the facile formation of the ultimate carcinogens **4a(b)** via N -> O transacylation from the corresponding hydroxamic acids **8a(b)** by base catalysis[7]!

Reactions of **4a(b)** with deoxyguanosine **7** in $CHCl_3/EtOH/H_2O$ (3:7:4) at 35°C in the presence of triethylamine led to the "adducts" **12**, **13** and **14**, which were separated by preparative HPLC[10].



12



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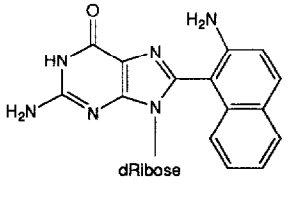

14

12 is identical with an adduct formed when hydroxylamine **5** was reacted with DNA *in vitro* at pH 5 followed by enzymatical splitting of the DNA[11]. The

same authors[11a] did not find the C-8 adduct **13**, however an adduct which is derived from **13** by hydrolytical ring cleavage between N-7 and C-8[12]. This adduct and **12** are also found in the epithelium of the bladder of dogs if they have been treated with 2-aminonaphthalene[13]. Because of the low yields of **14** we could not determine its structure definitively to date.

In summary, the isolation of the adducts **12**, **13** (and **14**) from reactions of N-acetoxy-2-aminonaphthalene **4a** with deoxyguanosine **7** is in agreement with the suggestion[1] that **4a** is in vivo an ultimate carcinogen. The reactions of **4a(b)** with N-methylaniline **6** support the strong electrophilic amination properties of these compounds.

Acknowledgement: This work has been supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the BASF AG.

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[2] T.J. Flammang, F.F. Kadlubar, Carcinogenesis 7 (1986) 919.

[3] see ref. [2] in: G. Boche, F. Bosold, S. Schröder, Angew. Chem. 100 (1988) 965; Angew. Chem. Int. Ed. Engl. 27 (1988) 973.

[4] The synthesis, isolation and complete characterization of N-acetoxy-4-aminobiphenyl and its reactions with deoxyguanosine are reported in M. Famulok, F. Bosold, G. Boche, Angew. Chem., submitted for publication.

[5] a. Preparation of **4a:** To a solution of 3.10 g (19.5 mM) **5** and 2.12 g (21.0 mM) triethylamine in 70 ml diethylether was added at -30°C 1.35 g (19.5 mM) acetylcyanide in 20 ml ether within 20 min under N_2 atmosphere. After 2h and warming to -20°C 60 ml ether were removed and 20 ml cold petrolether (40-60°C) added. The -20 to -30°C cold solution was filtered (filter: -78°C), diluted with 150 ml petrolether (40-60°C, -30°C cold) and cooled to -78°C for 2h. The yellow, very temperature sensitive crystals were separated and washed with petrolether (40-60°C) at -78°C, and dried at -30°C; yield: 2.32 g (59%). **4a** could not be characterized by means of elementary analysis and mass spectroscopy because of fast decomposition above 0°C.

NMR ($CDCl_3$, 230 K, 400 MHz) δ ^1H = 2.32 (s,3H), 7.23 (d, 1H), 7.42 (s,1H), 7.43 (t,1H), 7.51 (t,1H), 7.79 (d,1H), 7.83 (2d,2H), 9.01 (s,1H,NH). δ ^{13}C = 19.32, 111.64, 116.97, 124.73, 126.65, 127.02, 127.63, 129.01, 130.13, 133.28, 143.63, 170.82. IR (Nujol, -5°C, FT-IR) cm^{-1} = 3242, 1746, 1470, 1375, 1214, 818, 791.

b. Preparation of **4b:** **4b** was prepared analoguously to **4a**; yield 3.73 g (79%). NMR ($CDCl_3$, 230 K, 400 MHz) δ ^1H = 1.36 (s,9H), 7.24 (d,1H), 7.39

(s,1H), 7.43 (t,1H), 7.50 (t,1H), 7.78 (d, 1H), 7.82 (d,1H), 7.83 (d,1H), 9.09 (s,1H,NH). δ ^{13}C = 26.96, 38.40, 111.30, 116.84, 124.66, 126.62, 127.05, 127.64, 129.10, 130.07, 133.32, 144.10, 178.15. IR (Nujol, -5°C, FT-IR) cm^{-1} = 3203, 1743, 1466, 1377, 1119, 858, 750.

[6] Acylcyanides for O-acylation of arylhydroxylamines have first been used by S. Prabhakar, A.M. Lobo, M.M. Marques, Tetrahedron Lett. 23 (1982) 1391.

[7] see also: G. Boche, F. Bosold, S. Schröder, Angew. Chem. 100 (1988) 965; Angew. Chem. Int. Ed. Engl. 27 (1988) 973.

[8] **9**, **10** and **11** are characterized by elementary analysis, mass spectroscopy, ^{13}C and ^{1}H NMR spectroscopy; details of the preparation, separation and characterization will be given in the full paper.

[9] M. Famulok, F. Bosold, G. Boche, unpublished results.

[10] Details of the reactions and separation conditions will be given in the full paper (see also ref. [4]); the yields of **12**, **13** and **14** from **4a** and **4b** are 1.0%, 1.2% and 0.3%, respectively. We did not investigate the hydrolysis and decomposition products of **4a(b)**.

12: the ^{1}H NMR data agree with those given in Ref. [11a]. ^{13}C NMR (DMSO-d6, 400 MHz) δ = (in brackets: $^{1}J_{C-H}$ coupling constants, [Hz]): (the 2'-d-ribose C is covered by DMSO-signals), 61.66 (t, 140.7), 70.76 (d, 148.2), 82.34 (d, 165.12), 87.61 (d, 148.4), 111.25 (s), 117.41 (s), 118.88 (d, 158.6), 120.39 (d, 144.9), 121.13 (d, 161.8), 126.40 (d, 164.3), 126.87 (s), 127.94 (d, 152.1), 128.02 (d, 152.6), 132.44 (s), 135.69 (d, 213.9), 143.53 (s), 150.70 (s), 152.70 (s), 156.88 (s). IR (KBr), cm^{-1} = 3344, 1683, 1632, 1594, 1512, 1458, 1398, 1054. Mass spectr. (FD) m/z: 408 (M^+, 30%), 409 (M^+ +H, 100%), 431 (M^+ + Na, 65%).

13: NMR (DMSO-d6, 400 MHz) δ ^{1}H = 2.05 (m, 1H), 2.57 (m, 1H), 3.80 (m, 2H), 3.95 (m, 1H), 4.45 (m, 1H), 5.35 (s, 1H, OH), 6.02 (s, 1H, OH), 6.37 (q, 1H), 6.43 (s, 2H, NH_2), 7.31 (t,1H), 7.43 (t, 1H), 7.73-7.81 (4d, 4H), 8.34 (s, 1H), 8.84 (s, 1H, NH), 10.71 (s, 1H, NH). δ ^{13}C = (in brackets: $^{1}J_{C-H}$ coupling constants, [Hz]), 38.37 (t, 136.4), 61.21 (t, 141.7), 71.17 (d, 149.4), 82.77 (d, 163.1), 87.01 (d, 147.5), 111.63 (d, 162.9), 112.12 (s), 119.38 (d, 166.1), 123.27 (d, 160.5), 126.16 (d, 159.5), 126.51 (d, 159.9), 127.31 (d, 158.6), 127.94 (d, 160.6), 128.45 (s), 133.78 (s), 138.39 (s), 143.01 (s), 149.48 (s), 152.88 (s), 155.72 (s). IR (KBr), cm^{-1} = 3371, 1674, 1630, 1587, 1562, 1510, 1368, 1071. Mass spectr. (FD) m/z: 408 (M^+, 61%), 292 (M^+ - d-ribose +H, 100%).

14: NMR (DMSO-d6, 400 MHz) δ ^{1}H = 1.97 (m, 1H), 2.99 (m, 1H), 3.45 (m, 2H), 3.62 (m, 1H), 4.26 (m, 1H), 4.35* (1H), 4.99* (1H), 5.33* (2H), 5.55 (q, 1H), 5.73* (1H), 6.44* (2H), 7.08 (d, 1H), 7.09 (d, 1H), 7.16 (t, 1H), 7.27 (t, 1H), 7.72 (d, 1H), 7.76 (d, 1H). Mass spectr. (FD), m/z: 408 (M^+, 100%), 409 (52%) (*: exchanges with D_2O).

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(Received in Germany 7 November 1988)