SUPPORTING INFORMATION

Formation and Ecotoxicity of *N*-Heterocyclic Compounds on Ammoxidation of Mono- and Polysaccharides

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Synthesis of authentic samples

(5-Methyl-pyrazin-2-yl)methanol: 2.14 mL (2.16 g, 20 mmol) of 2,5-dimethylpyrazine was dissolved in 50 mL of CCl_4. 3.19 g (23.9 mmol) of *N*-chlorosuccinimide and 165 mg of 75% dibenzoylperoxide (123.6 mg, 0.68 mmol) were added portionwise, and the mixture was refluxed under argon for 8 h. GC/MS revealed a yield of 49% 2-chloromethyl-5-methylpyrazine, together with 16.5% of 2,5-bis(chloromethyl)pyrazine and 9% of unchanged educt. CCl_4 was then replaced by absolute ethanol, and 2.88 g (35.1 mmol) of sodium acetate was added. The mixture was refluxed under argon until > 95% of the chloride was substituted by acetate (44 h, control by GC/MS). After adding 0.72 g (18 mmol) of finely powdered sodium hydroxide, the mixture was refluxed for 15 min which quantitatively converted the acetates into the respective alcohols as confirmed by GC/MS. Then, the mixture was cooled to 0 °C, diluted with 80 mL of diethyl ether, and filtered. The filtrate was evaporated to dryness, and the remainder was purified by column chromatography (120 g silica, eluent EtOAc/CH_2Cl_2, 1:1, v/v) to afford 936 mg (38%) of the desired product and 143 mg (5.1%) of the by-product 2,5-bis(hydroxymethyl)pyrazine. As the product turns quickly brown at room temperature and light, it was stored in a dark place at 4 °C.

2-(*Arabino*-tetrahydroxybutyl)-5-(*erythro*-2,3,4-trihydroxybutyl)-pyrazine: To a solution of 1.00 g (25 mmol) of NaOH in 60 mL of water, 3.05 g (25 mmol) of phenylboronic acid was added and stirred until completely dissolved. D-Glucosamine hydrochloride (2.16 g, 10 mmol) was added portionwise over 5 min, and the solution was stirred at room temperature for 6 h. The consumption of glucosamine was monitored by thin layer chromatography (n-BuOH/AcOH/H_2O, v/v/v = 3:1:1, ninhydrin staining). The light yellow solution was acidified (pH ~2) by dropwise addition of 10% H_2SO_4, upon which phenylboronic acid precipitated quantitatively as off-white flakes which were removed by filtration. The aqueous phase was freeze-dried. 50 mL of methanol was added to the residue. After stirring at 40 °C for a few minutes, the solid fraction was separated by filtration and washed with a mixture of dry MeOH/EtOH (v/v = 1:1), until a small grain of the filter cake held into a flame did not char anymore. The filtrate was evaporated to dryness, dissolved in another 50 mL of methanol and filtered once more in order to remove any residual salt. Then, the solvent was evaporated and the residue dried under vacuum to afford 1.31 g (86%) of the desired product as a tan powder.

4-(Arabino-tetrahydroxybutyl)-imidazole: 5.40 g (30 mmol) D-Fructose and 5.03 g (36 mmol) formamidine acetate were loaded into a 100 mL glass liner. 20 mL of ammonia were

condensed into a trap cooled with dry ice/acetone, and subsequently transferred into the same glass liner, which was inserted into a Parr 4566 C pressure reactor, sealed, and stirred at 75 °C for 17 h. The reactor was cooled to dry ice temperature, opened, and allowed to warm up slowly to room temperature, upon which the ammonia evaporated. The residue was suspended in 50 mL of MeOH. Then the solution was evaporated to dryness and ammonia was stripped off for 6 h at reduced pressure (0.1 kPa, 40 °C). The remaining oil was dissolved in H_2O and loaded onto 40 g of strongly acidic ion exchange resin (Amberlite IRN77) to remove acetic acid. The loaded resin was washed with deionized H_2O until neutral, before the product was desorbed with 10% aqueous ammonia. The product fraction was evaporated to dryness under reduced pressure and the remaining crude product was then purified by column chromatography (Et_2O/MeOH, v/v = 2:1, containing 2% conc. ammonia) to give 2.04 g (36%) of an off-white powder.

EI-MS (70 eV) Fragmentation Pattern (Selection) of Persilylated Synthesized and Commercial *N*-Heterocyclic Compounds.

2-(Trimethylsilyloxymethyl)-5-methylpyrazine: 196 (M^+; 4.2%), 181 (M^+ -CH_3; 100%), 166 (M^+ –2 CH_3; 1.6%), 151 (M^+ - 3 CH_3; 2.2%), 107 (M^+ - $OSiCH_3$; 5.5%), 90 ($SiMe_3OH$; 1.5%), 80 (4.1%), 75 (13.7%), 73 ($SiMe_3$, 10.2%); of 2,5-bis(trimethylsilyloxymethyl)-pyrazine: 284 (M^+; 9.3%), 269 (M^+ - CH_3; 100%), 254 (M^+ - 2 CH_3; 4.2%), 195 (M^+ - $SiMe_3OH$; 2.7%), 179 (4.7%), 166 (1.0%), 147 ($(SiMe_3)O(Si^+Me_2)$; 1.6%), 127 (5.9%), 112 (1.2%), 106 (1.2%), 73 ($SiMe_3$; 12.9%).

2-(*Arabino*-1,2,3,4-tetra(trimethylsilyloxy)butyl)-5-(*erythro*-2,3,4-tris(trimethylsilyloxy) butyl)-pyrazine: 809 (M^+; 7.3%), 794 (M^+ - CH_3; 8.7%), 719 (M^+ - $SiMe_3OH$, 1.2%), 706 (M^+ –CH_2OSiMe_3; 2.2%), 604 (M^+ - $CH(OSiMe_3)CH_2OSiMe_3$; 3.9%), 574 (M^+ - $CH(OSiMe_3)CH_2OSiMe_3$ - C_2H_5; 28.1%), 502 (M^+ - $(CH(OSiMe_3))_2CH_2OSiMe_3$; 16.8%), 411 (6.4%), 307 ($^+(CH(OSiMe_3))_2CH_2OSiMe_3$; 71.0%), 217 ($^+CH_2CH(OSiMe_3)CH_2OSiMe_3$; 96.8%), 205 ($^+CH(OSiMe_3)CH_2OSiMe_3$; 13.5%), 103 ($^+CH_2OSiMe_3$; 78.8%), 73 ($^+SiMe_3$; 100%).

5-(*Arabino*-1,2,3,4-tetra-(trimethylsilyloxy)butyl)-1-trimethylsilyl-1*H*-imidazole): 548 (M^+; 1.7%), 533 (M^+ –CH_3; 3.5%), 443 (M^+ –CH_2OSiMe_3; 0.58%), 355 (2.8%), 344 (M^+ – $CH(OSiMe_3)CH_2OSiMe_3$; 1.1%), 329 (1.7%), 314 (1.9%), 255 (1.5%), 241 (M^+ – $(CH(OSiMe_3))_2CH_2OSiMe_3$; 100.%), 217 ($^+CH_2CH(OSiMe_3)CH_2OSiMe_3$; 1.4%), 147

((SiMe$_3$)O(Si$^+$Me$_2$); 5.5%), 103 ($^+$CH$_2$OSiMe$_3$; 2.3%), 73 ($^+$SiMe$_3$; 19.4%).

5-(*Arabino*-1,2,3,4-tetra-(trimethylsilyloxy)butyl)-1*H*-imidazole): 476 (M$^+$; 10.7%), 461 (M$^+$ –CH$_3$; 3.6%), 371 (M$^+$ –CH$_2$OSiMe$_3$; 2.5%), 329 (M$^+$ – (SiMe$_3$)O(SiMe$_2$); 1.5%), 307 ($^+$(CH(OSiMe$_3$))$_2$CH$_2$OSiMe$_3$; 4.4%), 283 (14.8%), 277 (4.4%), 257 (6.2%), 242 (M$^+$ – CH(OSiMe$_3$)CH$_2$OSiMe$_3$ – C$_2$H$_5$; 37.8%; assignment in analogy to (M$^+$ – 234)-fragment of per(trimethylsilyl)fructosazine),[1] 217 ($^+$CH$_2$CH(OSiMe$_3$)CH$_2$OSiMe$_3$; 25.0%), 169 (M$^+$ – (CH(OSiMe$_3$))$_2$CH$_2$OSiMe$_3$; 100%), 147 ((SiMe$_3$)O(Si$^+$Me$_2$); 23.1%), 103 ($^+$CH$_2$OSiMe$_3$; 41.1%).

Persilylated commercial *N*-heterocyclic compounds

1-Trimethylsilyl-4-methyl-1*H*-imidazole or 1-trimethylsilyl-5-methyl-1*H*-imidazole: 154 (77.8%), 139 (21.5%), 112 (26.0%), 98 (21.1%), 84 (7.0%), 73 (100%), 59 (6.2%).

1-Trimethylsilyl-2-methyl-1*H*-imidazole: 154 (M$^+$; 49.3%), 139 (M$^+$ - CH$_3$; 21.2%), 112 (9.5%), 98 (9.8%), 84 (4.6%), 73 ($^+$SiMe$_3$; 100%), 59 (6.1%).

1-Trimethylsilyl-4(5)-(trimethylsilyloxymethyl)-1*H*-imidazole: 242 (M$^+$; 0.03%), 227 (M$^+$ - CH$_3$; 0.3%), 170 (M$^+$ SiMe$_3$ +H; 14.6%), 155 (M$^+$ –Me -SiMe$_3$ +H; 100.0%), 125 (1.9%), 98 (1.8%), 81 (15.3%), 75 ($^+$SiMe$_2$OH; 79.7%), 73 ($^+$SiMe$_3$; 13.1%), 70 (4.7%), 54 (4.1%).

2-Trimethylsilyloxy-pyrazine: 168 (M$^+$; 19.4%), 153 (M$^+$ –CH$_3$; 100%), 137 (M$^+$ –2 CH$_3$; 1.7%), 123 (M$^+$ –3 CH$_3$; 2.2%), 98 (8.3%), 73 ($^+$SiMe$_3$; 5.5%).

Table S1: Peak Numbers, Retention Times and Peak Assignment for All Nitrogenous Compounds Detected in the Ethyl Acetate Extracts and Crude Ammoxidation Products of D-Glucose and D-Xylose.[2]

Compound number	Retention time [min]	Compound name
Amino sugars		
62	32.36	Glucosamine, tetrakis(TMS)[a]
62	33.29	Glucosamine, hexakis(TMS)
65	33.74	Fructosamine, hexakis(TMS)[b]
69	37.98, 38.17, 38.58, 39.31	Aminohexopyranosid, hexakis(TMS)[b]
71	41.15, 41.47, 41.76, 41.88, 42.01, 42.50, 46.26, 46.69, 47.10, 48.39, 49.57, 49.76, 49.94	Aminoglycosides of unknown constitution[b]
73	47.56	Di(glucopyranosyl)amine, octakis(TMS)[b]
Carboxylic acid amides		
86	7.16	Acetic amide[a]
6	15.03	Lactamide, bis(TMS)[a]
7	15.63	Glycolamide, bis(TMS)[a] (coeluting with pyrazinylmethanol)
10	17.25	Urea, N,N‘-bis(TMS)[a]
16	22.27	Glyceric amide, tris(TMS)[b]
32	8.61, 11.00	Bis(TMS) formamide (two peaks)[a]
33	8.95	Carbodiimide, N,N'-bis(TMS) (from urea)[a]
34	11.00, 15.95	Carbamate, bis(TMS) and -tris(TMS)[a]
39	17.51	Oxamic acid, bis(TMS)[b]
51	27.05	Trihydroxybutyric acid amide, tetrakis(TMS)[b]
59	32.00	Tetrahydroxypentonic acid amide, pentakis(TMS)[b]
α-Amino carboxylic acids		
41	13.56, 18.83	Glycine, bis(TMS) and -tris(TMS)[a]
47	23.20	2-Aminomalonic acid, tris(TMS)[d]
74	13.07	Alanine, bis(TMS)[a]
75	17.50, 20.43	Serine, bisTMS and –trisTMS[a]
1*H*-Imidazoles		
2	12.87	1*H*-Imidazole (TMS)[a]
5	14.68	4-Methyl-1*H*-imidazole (TMS)[a]
43	20.90	4-Hydroxymethyl-1*H*-imidazole, bis(TMS)[a]
55	28.79	4-(1,2-dihydroxyethyl)-1*H*-imidazole, tris(TMS)[b]
68	35.92	4-(D-Arabino-tetrahydroxybutyl)-1*H*-imidazole, pentakis(TMS)[a]
77	36.22	2-Acetyl-4-(tetrahydroxybutyl)-1*H*-imidazole, pentakis(TMS)[b]

Pyridines

4	13.90	3-Hydroxypyridine, TMS[a]
20	24.60	2-(Hydroxymethyl)-pyridin-5-ol, bis(TMS)[b]

Pyrazines

1	11.85	2-Pyrazinol, TMS[a]
3	13.02	2-Hydroxy-5-methylpyrazine, TMS or isomer[b]
7	15.61	2-Pyrazinylmethanol, TMS[a]
8	15.83	2-Hydroxy-3-methylpyrazine, TMS or isomer[b]
12	17.94	2-Hydroxymethyl-6-methylpyrazine, TMS[a]
18	23.45	2-(Dihydroxyethyl)pyrazine, bis(TMS)[c]
21	24.77, 25.10, 25.36, 25.83, xylose only: 24.23	2-(Dihydroxyethyl)-5-methyl-pyrazine, bis(TMS)[b] and isomers
22	26.34, 26.57	2,5[a]- and 2,6[b]-Bis(hydroxymethyl)pyrazine, bis(TMS)
23	31.76	2-(Dihydroxyethyl)-5(hydroxymethyl)pyrazine, tris(TMS)[b]
24	33.07	2-(Tetrahydroxybutyl)pyrazine, tetrakis(TMS)[b]
25	33.45, 33.60, 33.75, 33.90	2-(Tetrahydroxybutyl)-5-methyl-pyrazine, tetrakis(TMS)[b] and isomers
28	28.76, 28.91	2-(Trihydroxypropyl)pyrazine, tris(TMS)[b], 2 isomers
29	29.48, 29.58, 29.76, 29.86	2-(Trihydroxybutyl)-5-methylpyrazine, tris(TMS) and isomers[b]
30	34.90, 35.50	2-(Trihydroxybutyl)-5-(hydroxymethyl)pyrazine, tetrakis(TMS) and isomers[b]
78	36.10, 36.34	2-Hydroxy-5 (and 6)-(tetrahydroxybutyl)pyrazine, pentakis(TMS)[b]
79	37.96, 38.36	2-(Hydroxymethyl)-5 (and 6)-(tetrahydroxybutyl)pyrazine, pentakis(TMS)[b]
80	39.24, 39.79	2-(2-Hydroxyethyl)-5 (and 6)-tetrahydroxybutyl pyrazine, pentakis(TMS)[b]
81	41.23, 41.58	2-(Dihydroxyethyl)-5 (and 6)-tetrahydroxybutyl pyrazine, hexakis(TMS)[b]
82	42.92, 43.37	2-(2,3-Dihydroxypropyl)-5 (and 6)-tetrahydroxybutyl pyrazine, hexakis(TMS)[b]
83	43.92, 44.40	2-(Trihydroxypropyl)-5 (and 6)-tetrahydroxybutyl pyrazine, hexakis(TMS)[b]
84	46.03, 46.08	2,5- and 2,6-Deoxyfructosazine, heptakis(TMS)[b]
85	46.87, 47.11,48.07,48.28	2,5- and 2,6-Fructosazine and diastereomers, octakis(TMS)[b]

[a]) identified by comparison with authentic samples; [b]) assignment according to fragmentation pattern; [c]) Patey 1986[3]; [d]) Tsuchida.[1, 4]

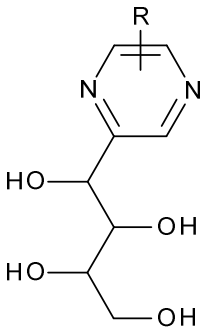
Table S2: Nitrogen Contents of the Water-Soluble and Water-Insoluble Phases Obtained by Ammoxidation (3 h) of Cellulose, Xylan, D-Glucose and D-Xylose.

	pressure [MPa]	r.t.	70 °C	100 °C	140 °C
			[N content %]		
Cellulose	0.2	0.07[a]	0.08	0.10	0.16
(insoluble part)	1.0		0.10	0.09	0.18
D-Glucose	0.2	0.44[a]	13.5	13.3	13.5
(soluble part)	1.0		5.3	5.2	16.3
Xylan	0.2	0.49[a]	0.39	0.57	0.81
(insoluble part)	1.0		0.41	0.60	1.70
Xylan	0.2	0.38[a]	0.52	2.76	9.95
(soluble part)	1.0		0.48	1.85	n.d.[b]
D-Xylose	0.2	2.69[a]	8.2	15.7	17.7
(soluble part)	1.0		9.2	n.d.[b]	20.0

[a]) ambient pressure [b]) not determined

Table S3: Ammoxidation (0.2 MPa O_2, 3 h) of D-Glucose: Temperature-Dependent Formation of 2-(1,2,3,4-Tetrahydroxybutyl)-pyrazine Derivatives Carrying a (Poly)hydroxyalkyl Substituent of Different Chain Length (n=0-4) at 5 or 6 Position of the Heterocyclic Ring.

chain length[a]	R=	70 °C	100 °C	140 °C
0	H	0.11	2.66	0.50
1	CH_3 CH_2OH	0.18	4.26	2.12
2	CH_2CH_2OH $(CHOH)CH_2OH$	0.02	0.95	0.81
3	$CH_2(CHOH)_2H$ $(CHOH)_3H$	0.02	0.11	0.05
4	$CH_2(CHOH)_3H$ $(CHOH)_4H$	0.05	0.86	0.45



[a]) The given values were calculated as ratio of the cumulated peak areas for all pyrazine derivatives of one particular chain length of the substituent in C5(6) position and that obtained from 200 µg of the internal standard phenyl glucoside.

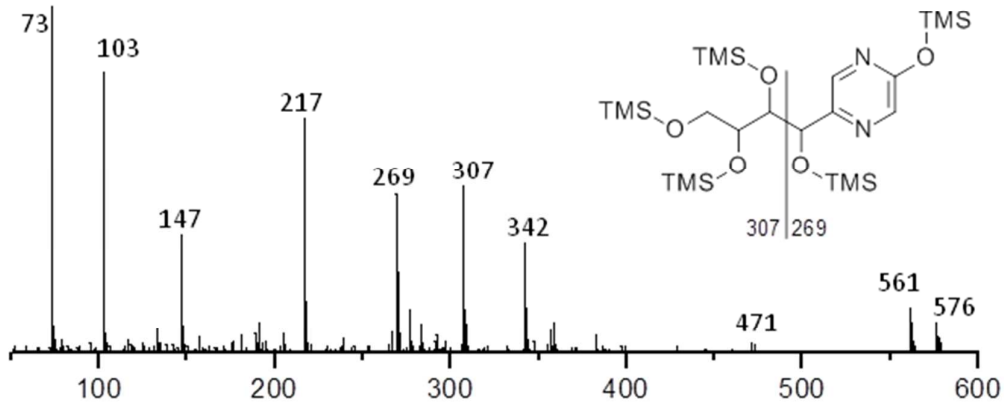
Table S4: Growth Inhibition of *Pseudokirchneriella subcapitata* at 0.5 g/L Sample Concentration (= Maximum Concentration) for Ammoxidized (0.2 MPa O_2, 3 h) D-Glucose and D-Xylose (Crude Reaction Mixtures).

Compound	Inhibition at 0.5 g/L in %
D-Glucose (100 °C)	68
D-Glucose (140 °C)	35
D-Xylose (100 °C)	26

Table S5: pH of Ammoxidation Mixtures After 3 h of Reaction Time (5% NH_3, 0.2 MPa O_2, 50 g/L Substrate, starting pH = 11.9).

	70 °C	100 °C	140 °C
Cellulose	11.1	11.3	10.8
D-Glucose	10.5	10.1	9.9
Xylan	11.2	10.6	10.4
D-Xylose	10.0	10.0	10.0

Figure S1: EI-MS (70 eV) spectrum and suggested structure of peak 78 (penta(TMS)-2-hydroxy-5(6)-tetrahydroxybutyl pyrazine).



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