SUPPLEMENTAL MATERIAL

Selective utilization of benzimidazolyl-norcobamides as cofactors by the tetrachloroethene reductive dehalogenase of *Sulfurospirillum multivorans*

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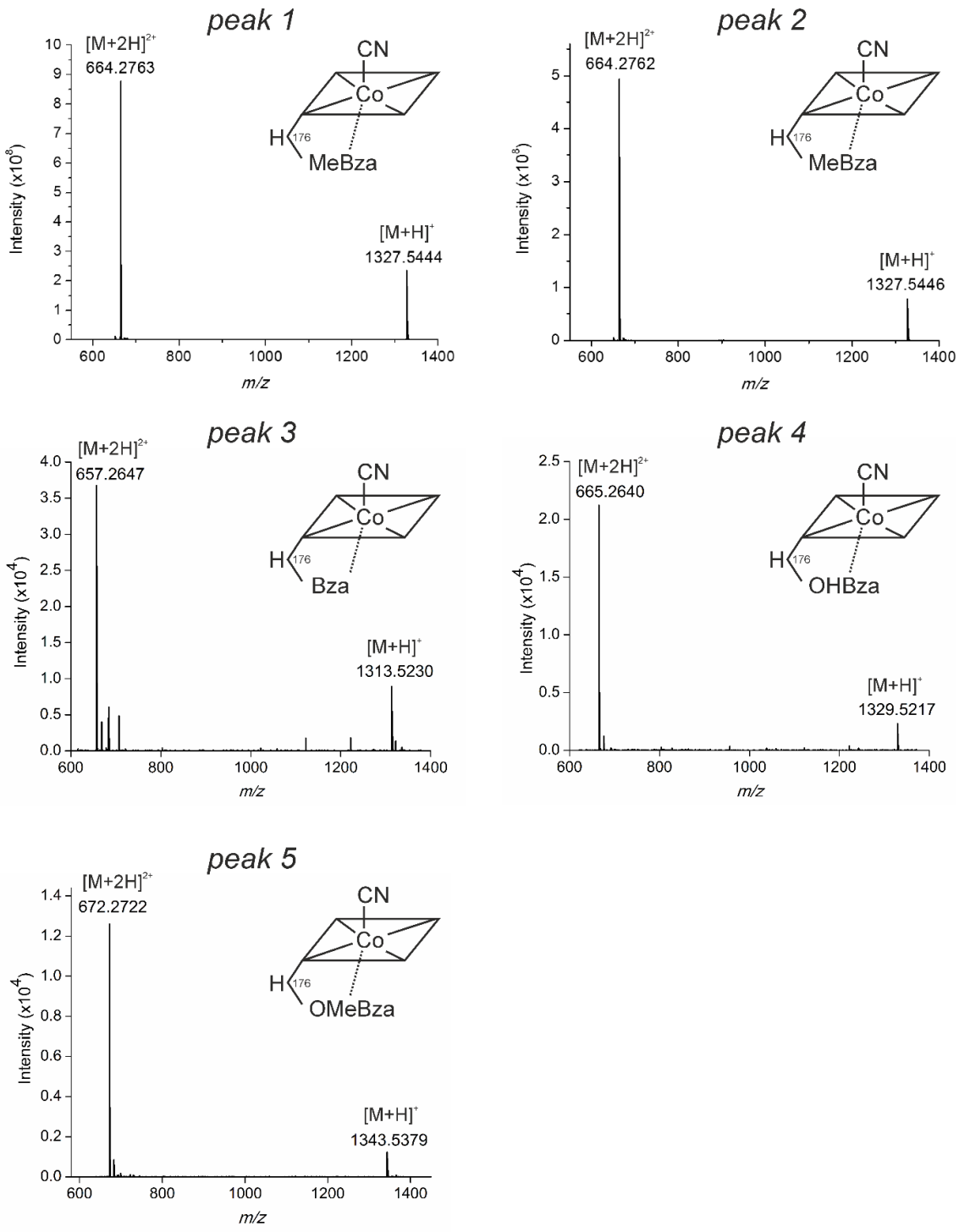

Fig. S1: Data from UHPLC-ESI-HRMS analysis of purified NCbas. Singly and doubly protonated ions were detected, which were assigned to the respective NCbas. Numbering of the peaks is referring to figure 1C.

Tab. S1: Calculated monoisotopic masses of the NCbas in comparison to the measured values. Labeling of the peaks is in accordance to figure 1C.

	Compound	Accurate mass (*m/z*)	Calculated mass (*m/z*)	ƍ (ppm)	Elemental composition
Peak 1					
$[M+H]^+$	MeBza-NCba	1327.5444	1327.5434	0.752	$C_{61}H_{85}O_{14}N_{14}CoP$
$[M+2H]^{2+}$		664.2763	664.2753	1.477	$C_{61}H_{86}O_{14}N_{14}CoP$
Peak 2					
$[M+H]^+$	MeBza-NCba	1327.5446	1327.5434	0.888	$C_{61}H_{85}O_{14}N_{14}CoP$
$[M+2H]^{2+}$		664.2762	664.2753	1.266	$C_{61}H_{86}O_{14}N_{14}CoP$
Peak 3					
$[M+H]^+$	Bza-NCba	1313.5230	1313.5277	-3.602	$C_{60}H_{83}O_{14}N_{14}CoP$
$[M+2H]^{2+}$		657.2647	657.2675	-4.205	$C_{60}H_{84}O_{14}N_{14}CoP$
Peak 4					
$[M+H]^+$	OHBza-NCba	1329.5218	1329.5227	-0.606	$C_{60}H_{83}O_{15}N_{14}CoP$
$[M+2H]^{2+}$		665.2640	665.2650	-1.460	$C_{60}H_{84}O_{15}N_{14}CoP$
Peak 5					
$[M+H]^+$	OMeBza-NCba	1343.5379	1343.5383	-0.429	$C_{61}H_{85}O_{15}N_{14}CoP$
$[M+2H]^{2+}$		672.2722	672.2728	-0.857	$C_{61}H_{86}O_{15}N_{14}CoP$
#					
$[M+H]^+$	norpseudo-B_{12}	1330.5283	1330.5291	-0.607	$C_{58}H_{82}O_{14}N_{17}CoP$

NMR data (extended)

Structure analysis of the 6-OHBza-*N*-α-ribofuranosyl moiety of 6-OHBza-norcobamide. All norcobamide (NCba) structures were analyzed by NMR spectroscopy with focus on the various benzimidazoles incorporated during the cultivation experiments. In the following, the structure analysis of the 6-OHBza-*N*-α-ribofuranosyl moiety of 6-OHBza-NCba is described as a typical example (Fig. S2-9). In an analogous manner the structures and chemical shifts of the 5-OMeBza-NCba (Fig. S10-15), the 5-MeBza-NCba (Fig. S16-21), and the 6-MeBza-NCba (Fig. S22-27) have been worked out.

The ^{1}H NMR spectrum showed six signals in the low field range, three of them attributable to an aromatic AMX spin system. However, a deviation of the chemical shifts compared to the literature (Crofts *et al.*, 2014) was observed and thus the structure was elucidated *de novo*. Starting point was a ^{1}H-^{13}C HSQC cross signal at δ_H 6.17, *m*/δ_C 86.6 (Fig. S7), which was assigned to position 1' of the α-ribofuranose moiety. By means of ^{1}H-^{1}H DQFCOSY, selective ^{1}H-^{1}H TOCSY (offset on H-1') and ^{1}H-^{13}C HSQC all remaining positions of the α-ribofuranose were assigned (Fig. S5) The ^{1}H-^{13}C HMBC (Fig. S8) correlation of H-1' with C-2 (δ_C 141.8) was proving the connection of the α-ribofuranosyl with the benzimidazolyl part. In order to elucidate the position of the hydroxyl group at the benzimidazole, a ^{1}H-^{1}H ROESY experiment was conducted (Fig. S9). Correlation of H-1' with the broad singlet of H-7 (δ_H 6.80) was observed, and ^{1}H-^{13}C HSQC revealed the corresponding C-7 at δ_C 96.8. Furthermore, H-4' (δ_H 4.02, *m*) showed a ROE-correlation to H-2 (δ_H 7.03), indicating that the α-ribofuranosyl and benzimidazolyl systems have a perpendicular orientation towards each other. Because no ^{1}H-^{1}H DQFCOSY correlation of H-7 was observed, the

position of the hydroxyl group in the benzimidazolyl moiety had to be in position 6. The remaining protonated positions of the benzimidazole were assigned from ^{1}H-^{13}C HMBC and ^{1}H-^{1}H DQFCOSY correlations, respectively. ^{1}H-^{13}C HMBC correlations from H-7 revealed the chemical shifts of position 5 (δ_H 6.73, *d*, $^{3}J_{HH}$=8.9 Hz /δ_C 113.2) and position 9 (δ_C 132.2). H-4 (δ_H 6.49, *d*, $^{3}J_{HH}$=8.9 Hz) was determined from a ^{1}H-^{1}H DQFCOSY correlation with H-5. The corresponding ^{13}C chemical shift C-4 (δ_C 117.4) was determined by ^{1}H-^{13}C HSQC. The ^{13}C chemical shifts of position 8 (δ_C 132.5) and 6 (δ_C 153.7) were determined from the ^{1}H-^{13}C HMBC correlation with H-4. Finally, the ^{1}H-^{13}C HMBC correlation of H-2 with C-8 and C-9, the evidence for the linkage of the imidazolyl moiety with the phenyl moiety of benzimidazole, completed the structure elucidation of the 6-OHBza-NCba.

References:

Crofts TS, Hazra AB, Tran JL, Sokolovskaya OM, Osadchiy V, Ad O, Pelton J, Bauer S, Taga ME. 2014. Regiospecific formation of cobamide isomers is directed by CobT. Biochemistry. 53:7805-7815.

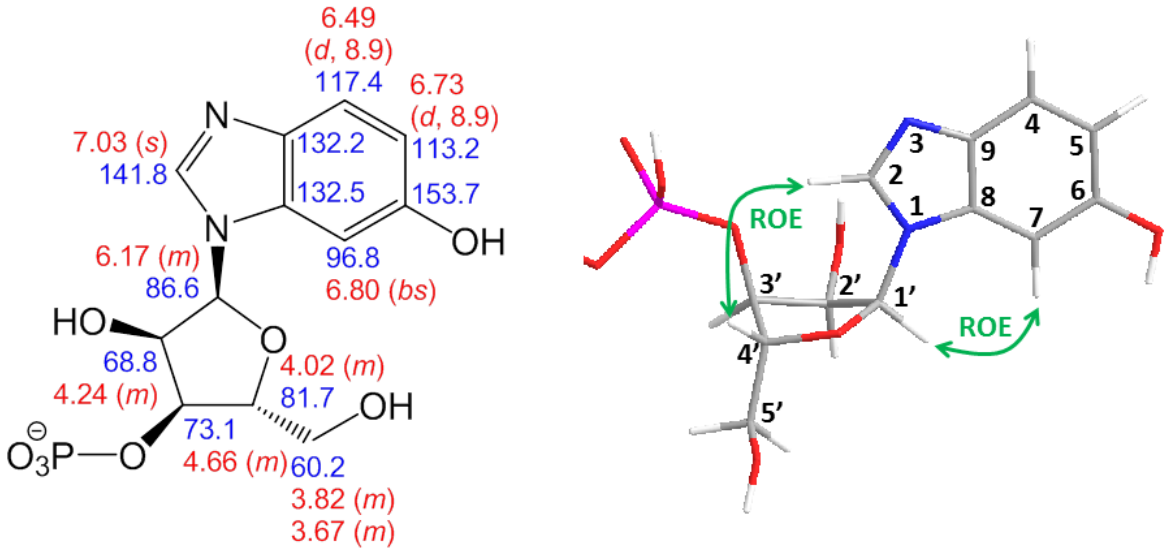

Fig. S2: Chemical shifts (δ_H red, δ_C blue) of the 6-OHBza-*N*-α-ribofuranosyl-fragment and the ^{1}H-^{1}H ROESY key correlations for determination of the position of the hydroxyl group.

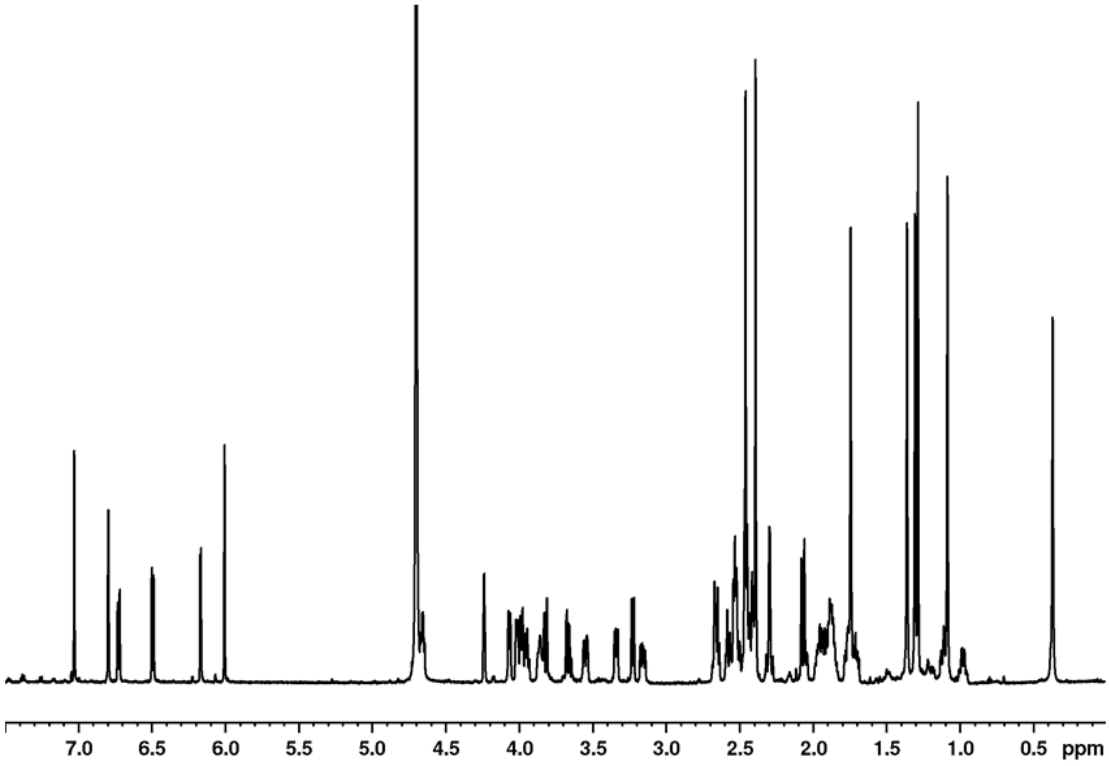

Fig. S3: ^{1}H-NMR spectrum of the 6-OHBza-NCba.

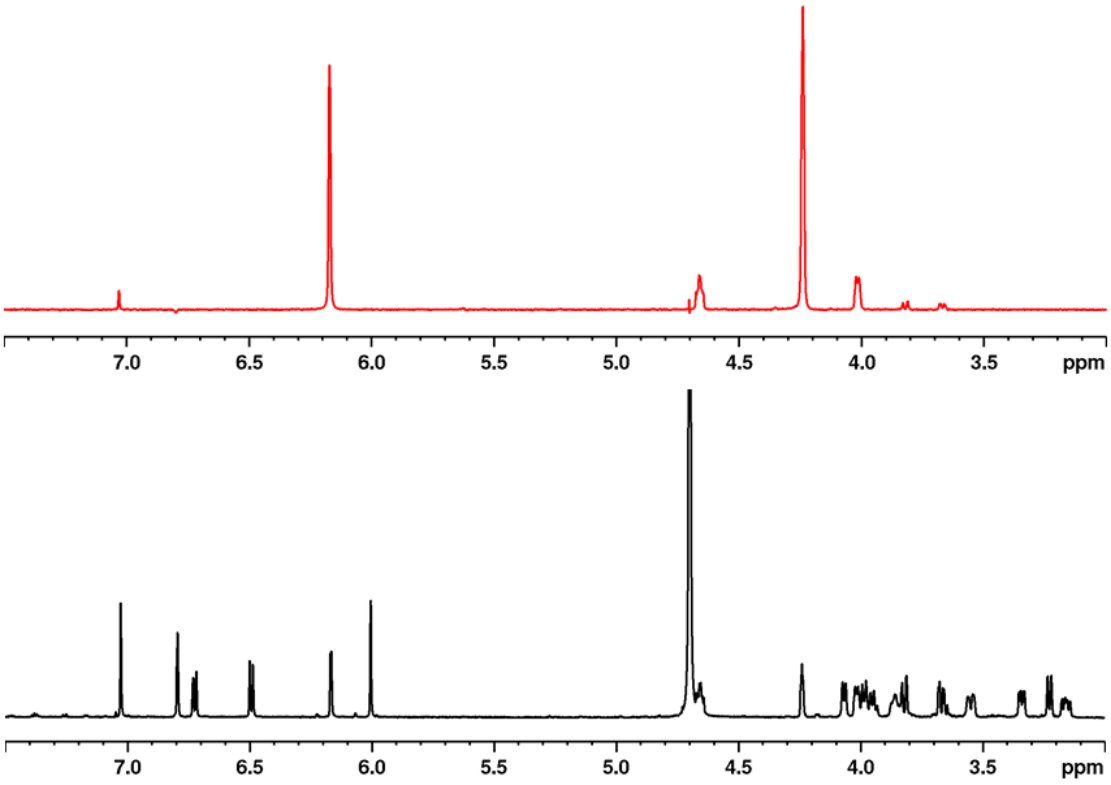

Fig. S4: ^{1}H-SELTOCSY spectrum of the α-ribofuranosyl moiety (red) of the 6-OHBza-NCba compared to the ^{1}H-NMR spectrum (black) of the 6-OHBza-NCba.

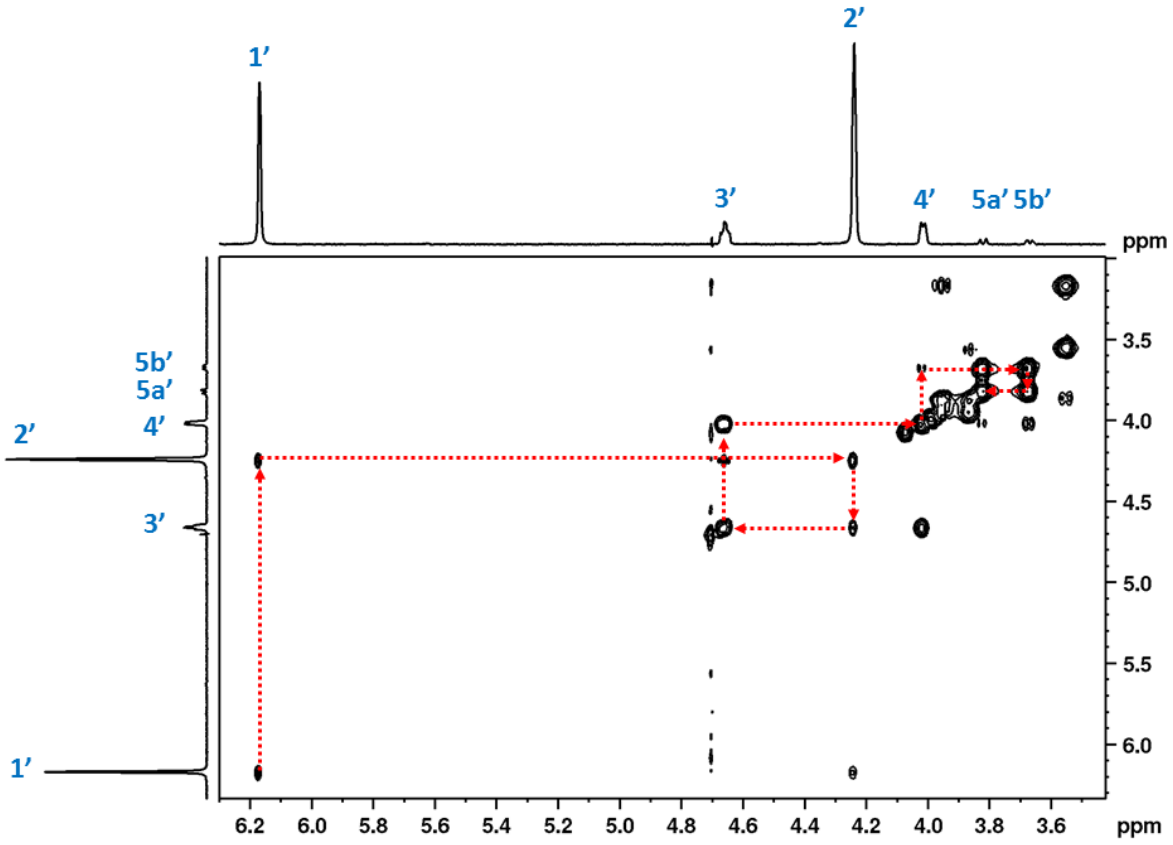

Fig. S5: ^{1}H-^{1}H DQFCOSY spectral detail of the α-ribofuranosyl unit of 6-OHBza-NCba with SELTOCSY spectra as projections.

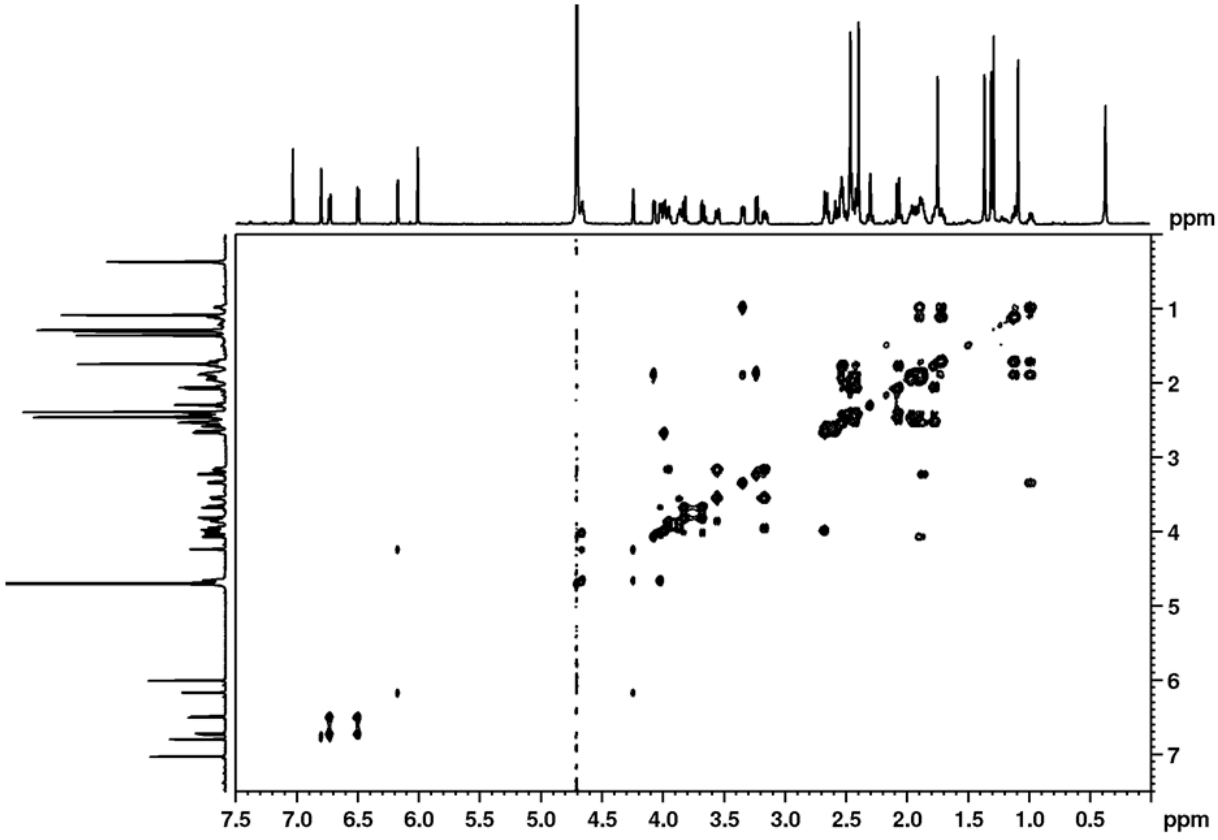

Fig. S6: Full ^{1}H-^{1}H-DQFCOSY spectrum of the 6-OHBza-NCba.

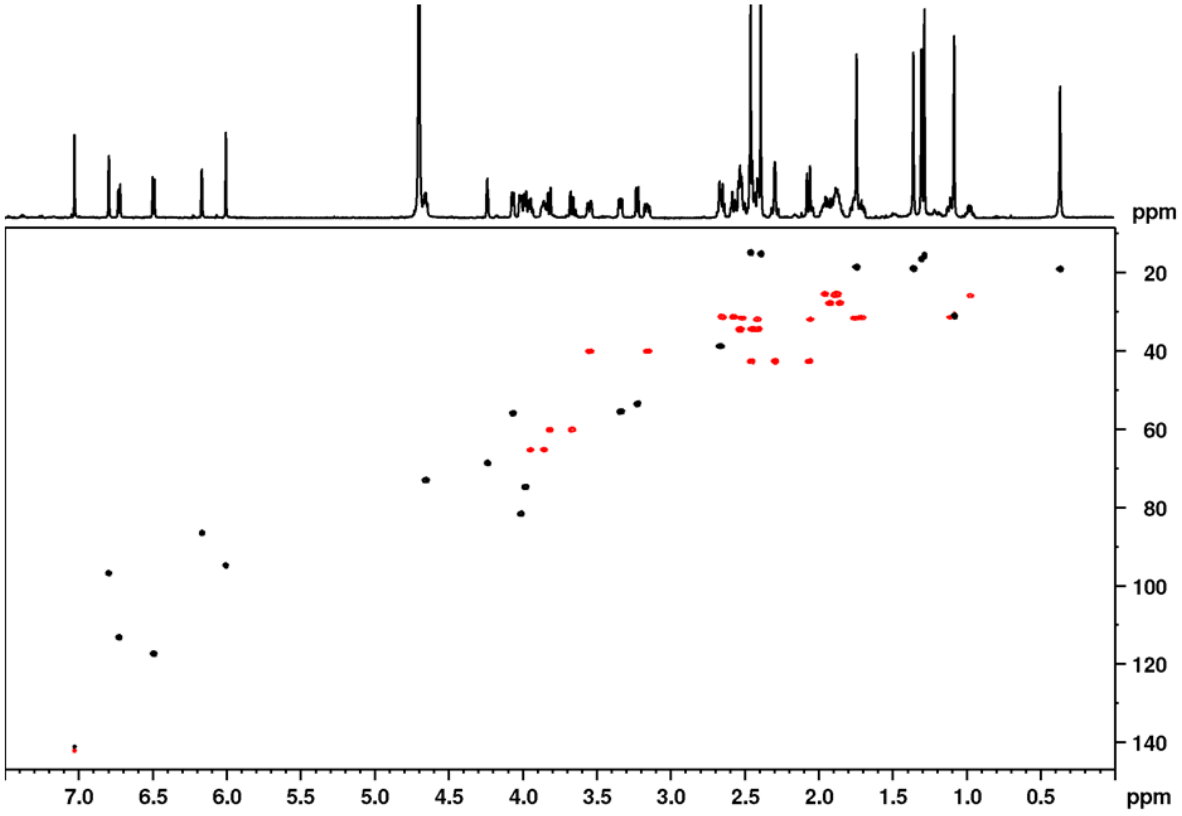

Fig. S7: ^{1}H-^{13}C HSQC spectrum of 6-OHBza-NCba.

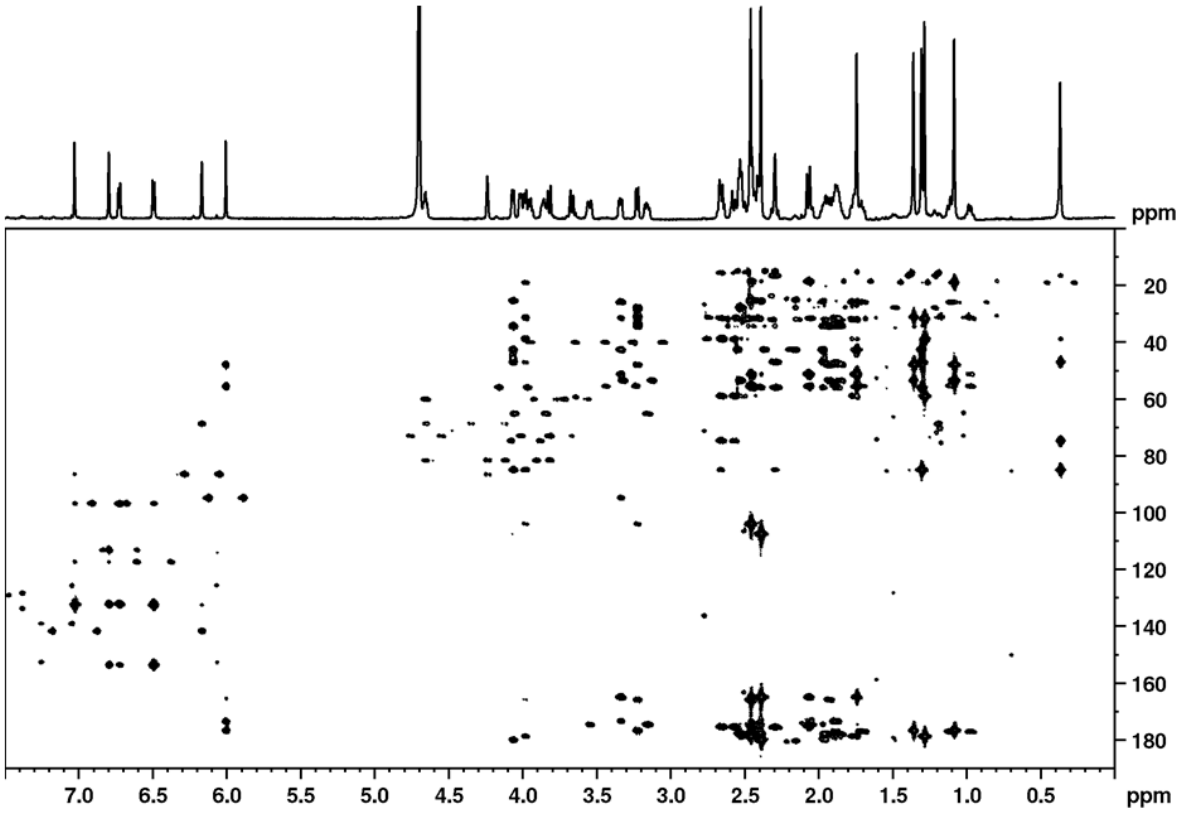

Fig. S8: ^{1}H-^{13}C HMBC spectrum of the 6-OHBza-NCba.

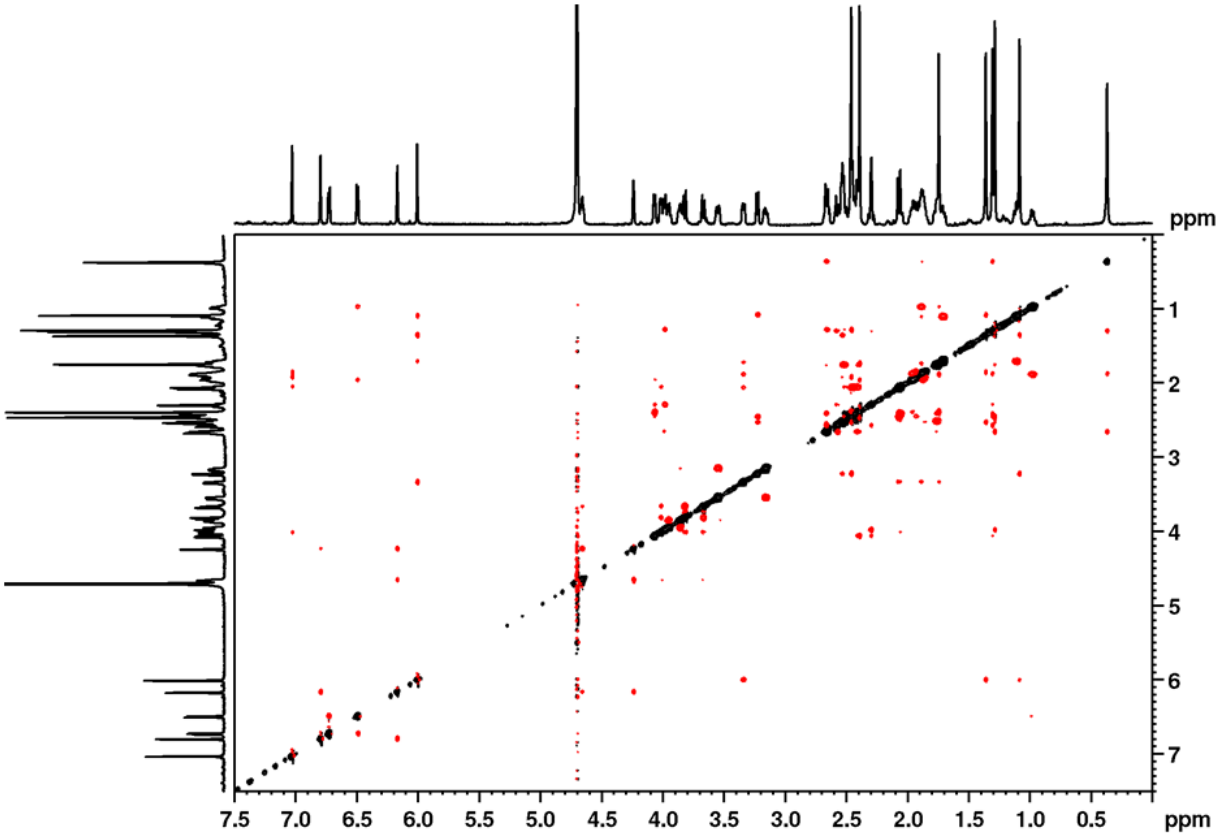

Fig. S9: ^{1}H-^{1}H ROESY spectrum of the 6-OHBza-NCba.

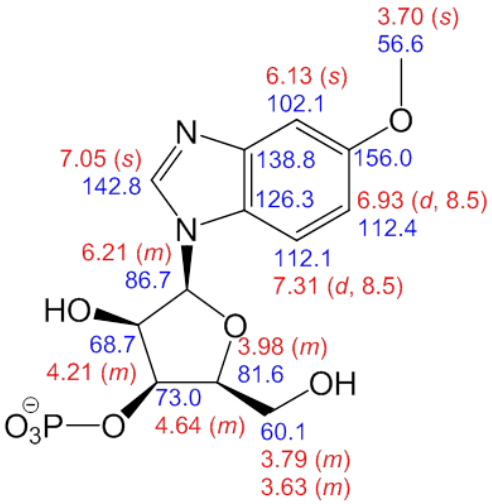

Fig. S10: Chemical shifts (δ_H red, δ_C blue) of the 5-OMeBza-*N*-α-ribofuranosyl-fragment.

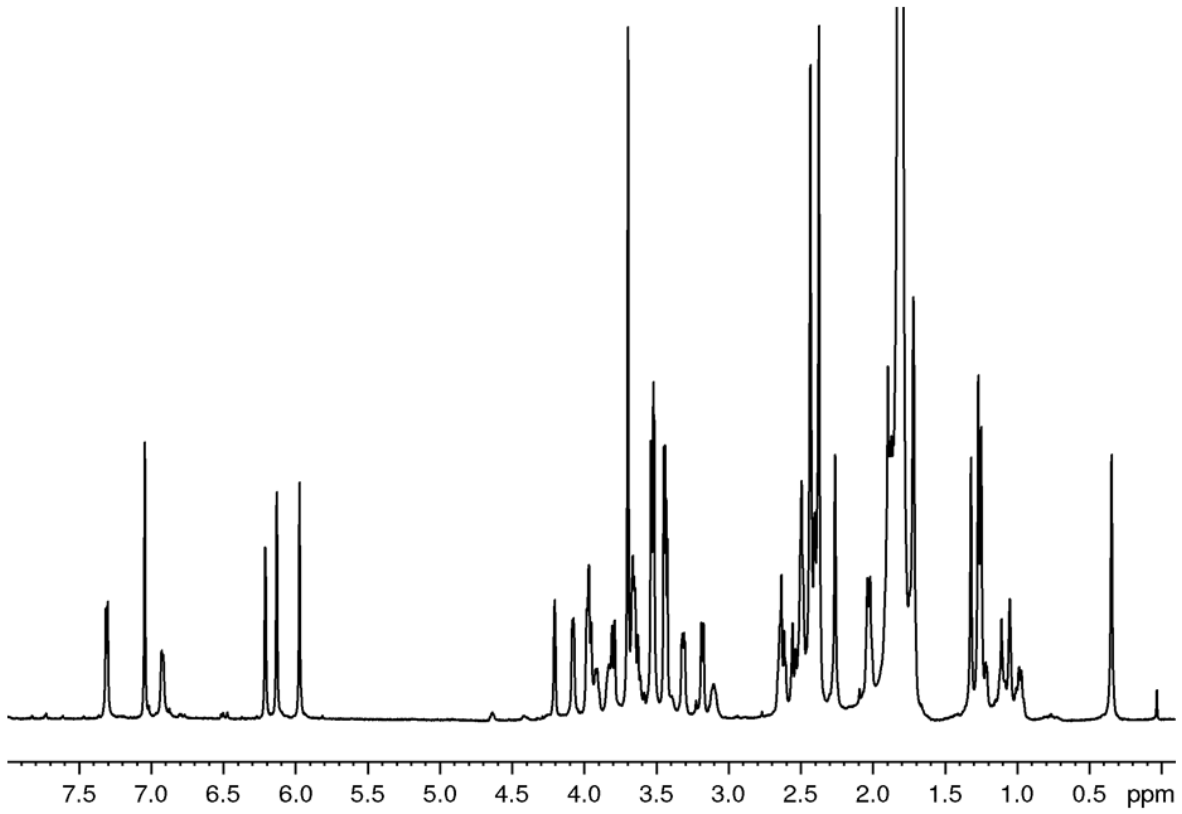

Fig. S11: ^{1}H-NMR spectrum of the 5-OMeBza-NCba.

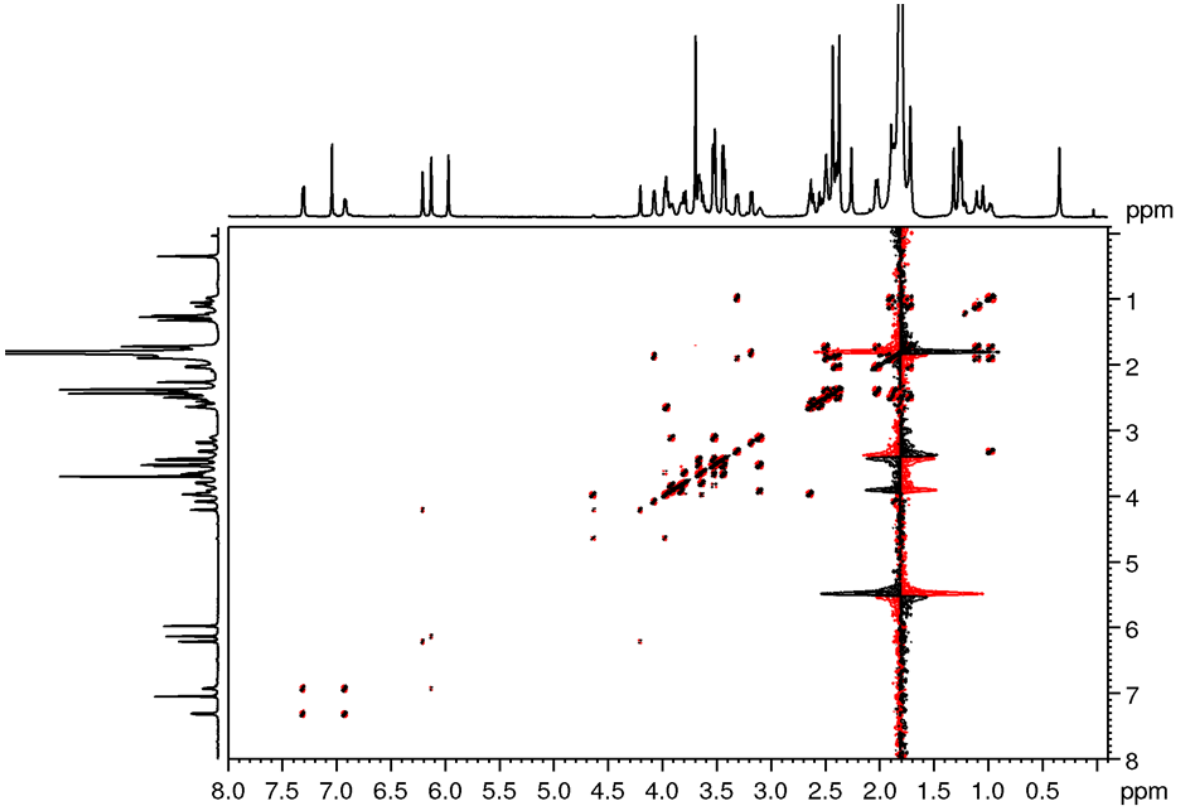

Fig. S12: ^{1}H-^{1}H-DQFCOSY spectrum of the 5-OMeBza-NCba.

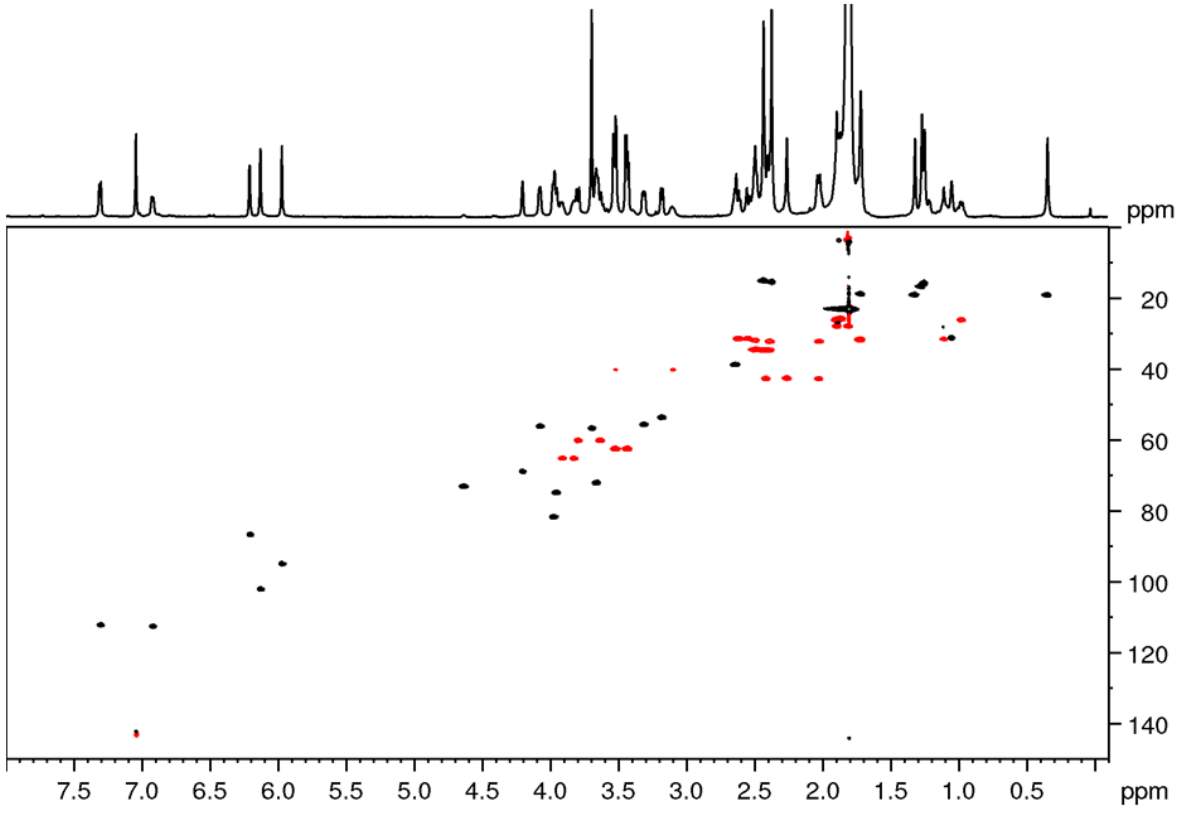

Fig. S13: ^{1}H-^{13}C HSQC spectrum of 5-OMeBza-NCba.

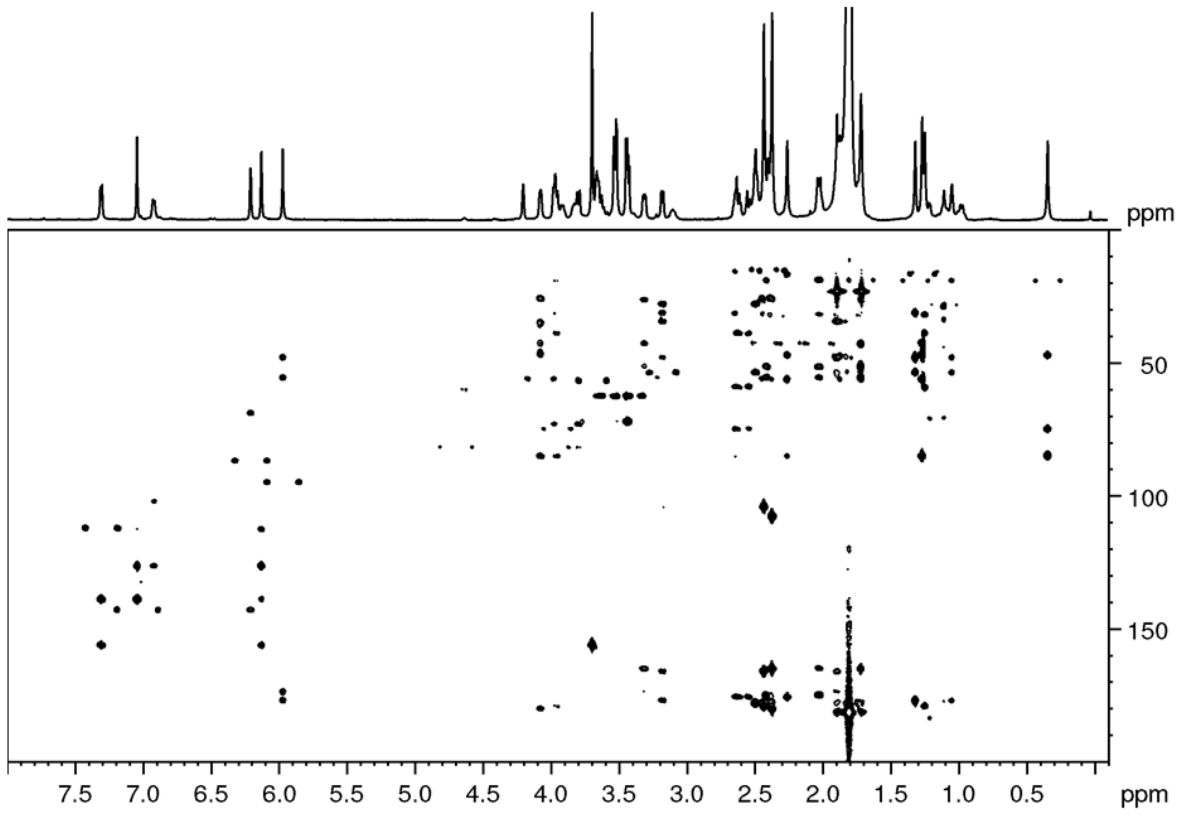

Fig. S14: ^{1}H-^{13}C HMBC spectrum of the 5-OMeBza-NCba.

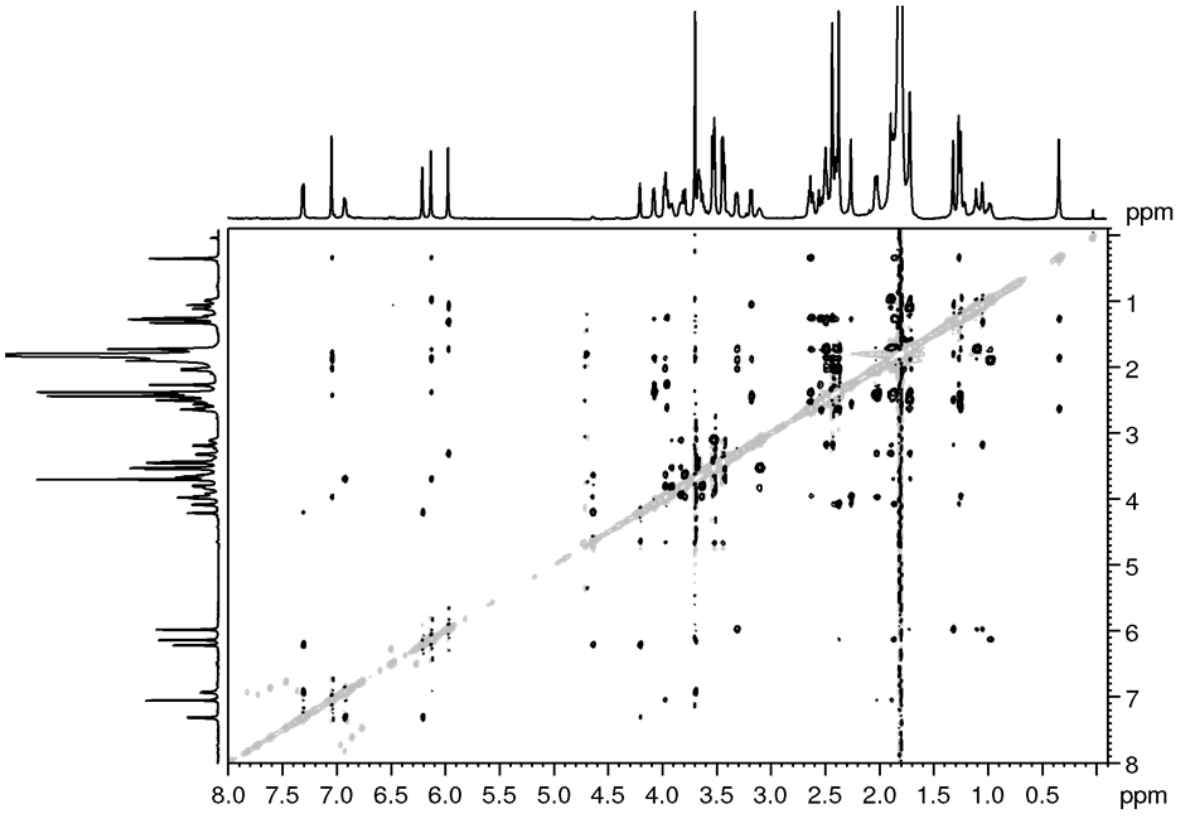

Fig. S15: ^{1}H-^{1}H ROESY spectrum of the 5-OMeBza-NCba.

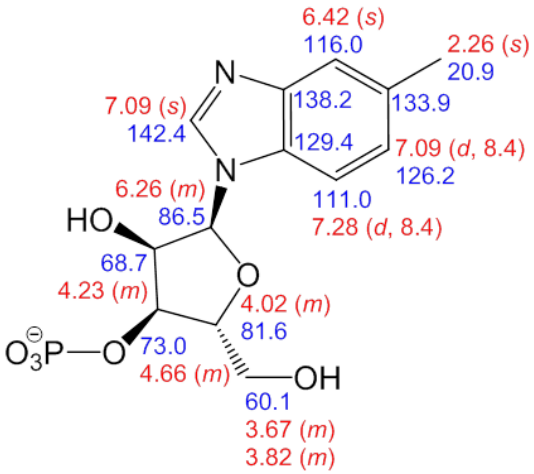

Fig. S16: Chemical shifts (δ_H red, δ_C blue) of the 5-MeBza-*N*-α-ribofuranosyl-fragment.

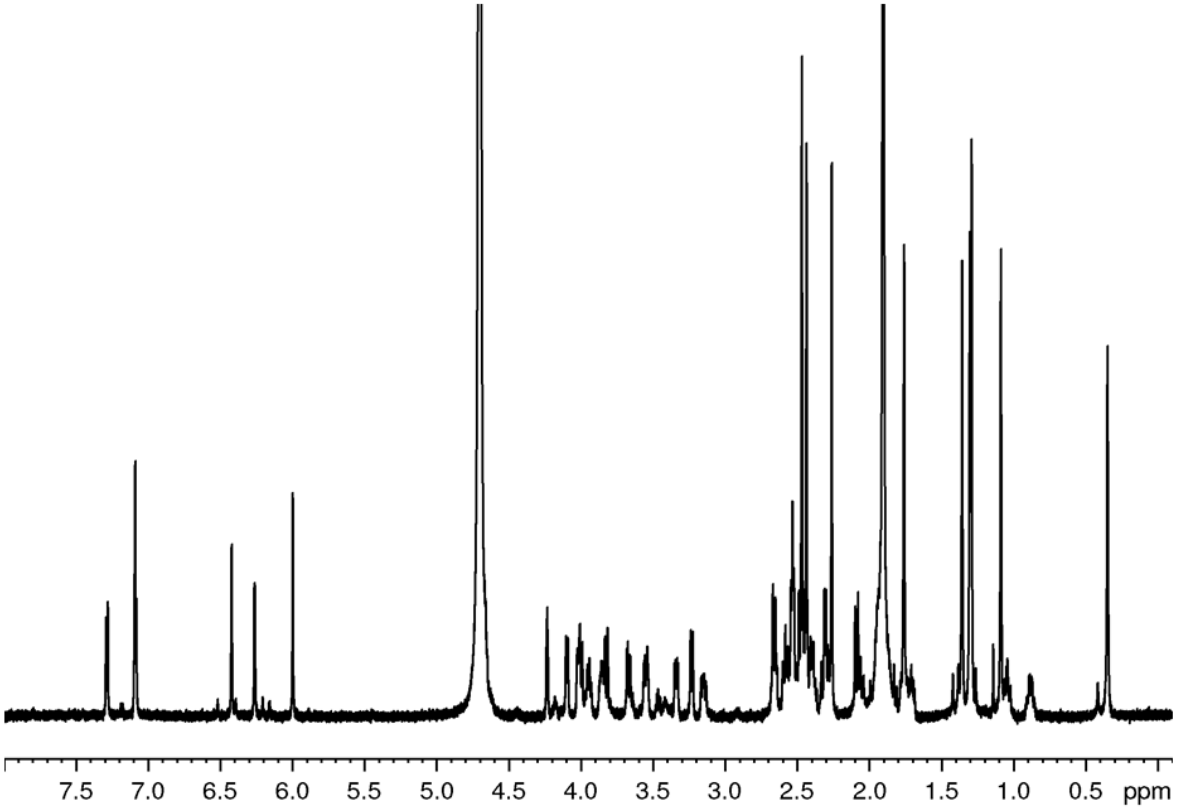

Fig. S17: ^{1}H-NMR spectrum of the 5-MeBza-NCba.

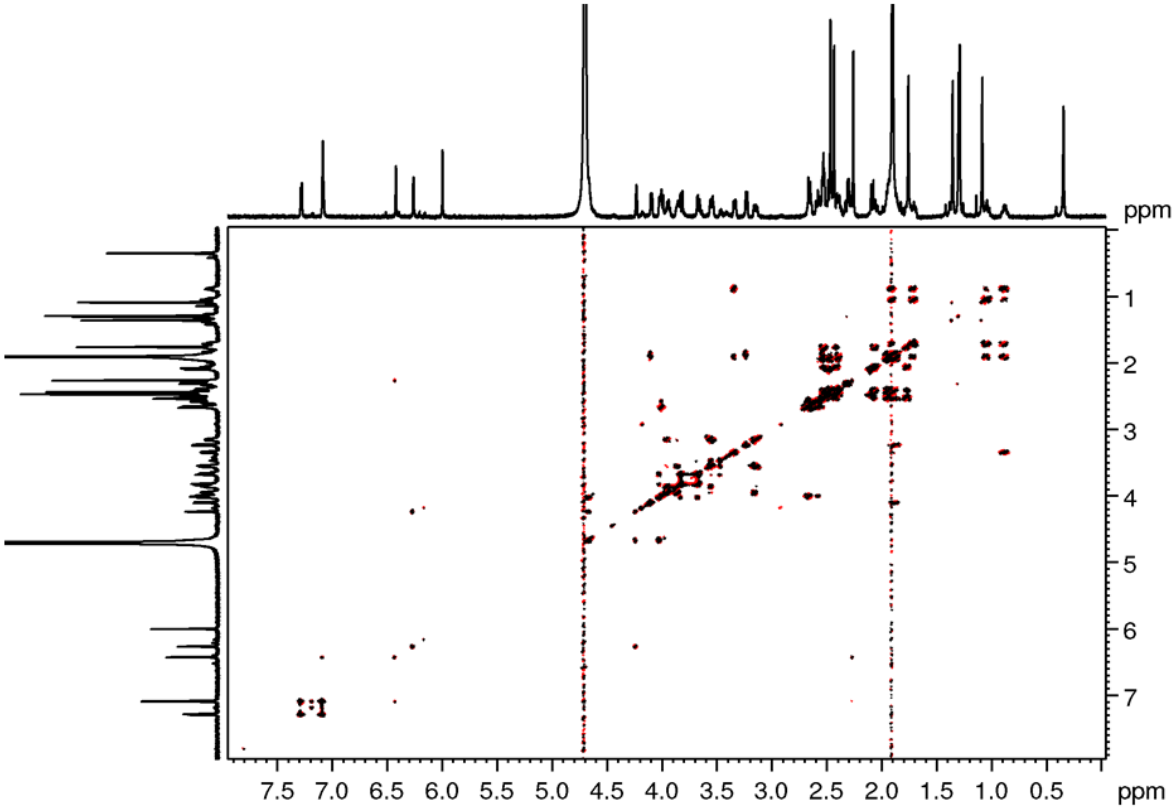

Fig. S18: ^{1}H-^{1}H-DQFCOSY spectrum of the 5-MeBza-NCba.

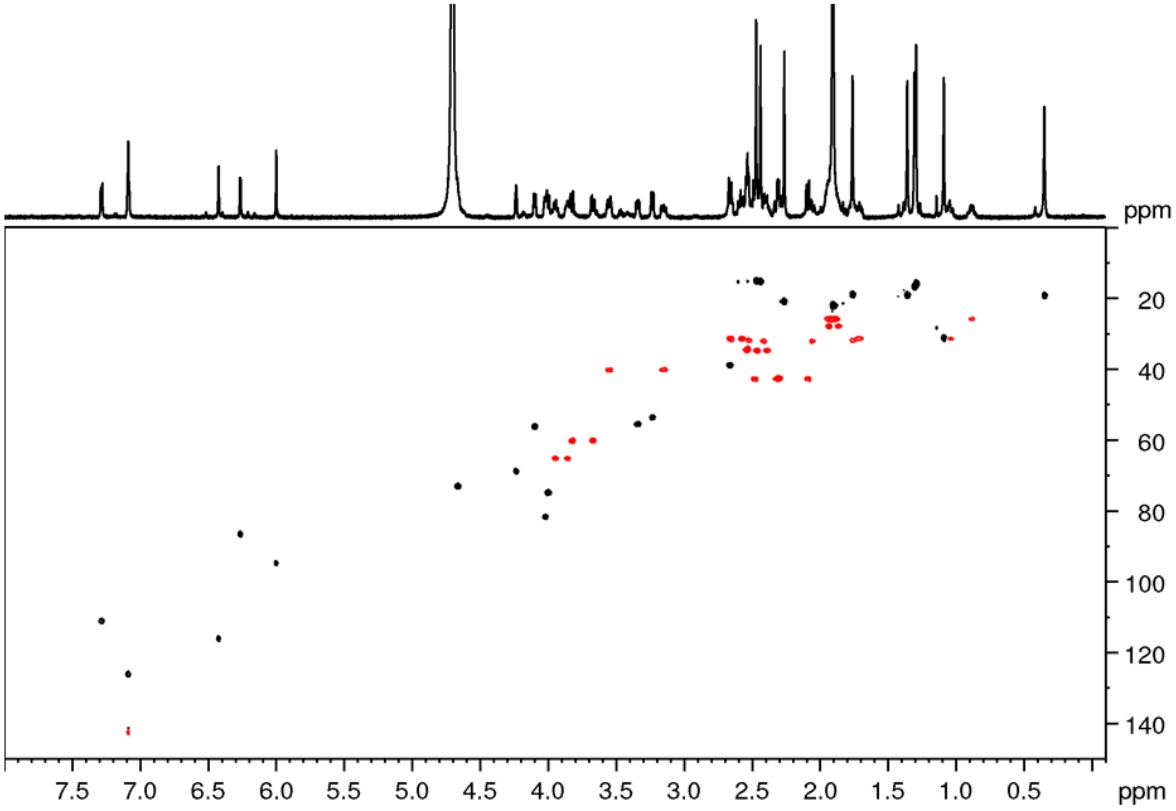

Fig. S19: ^{1}H-^{13}C HSQC spectrum of 5-MeBza-NCba.

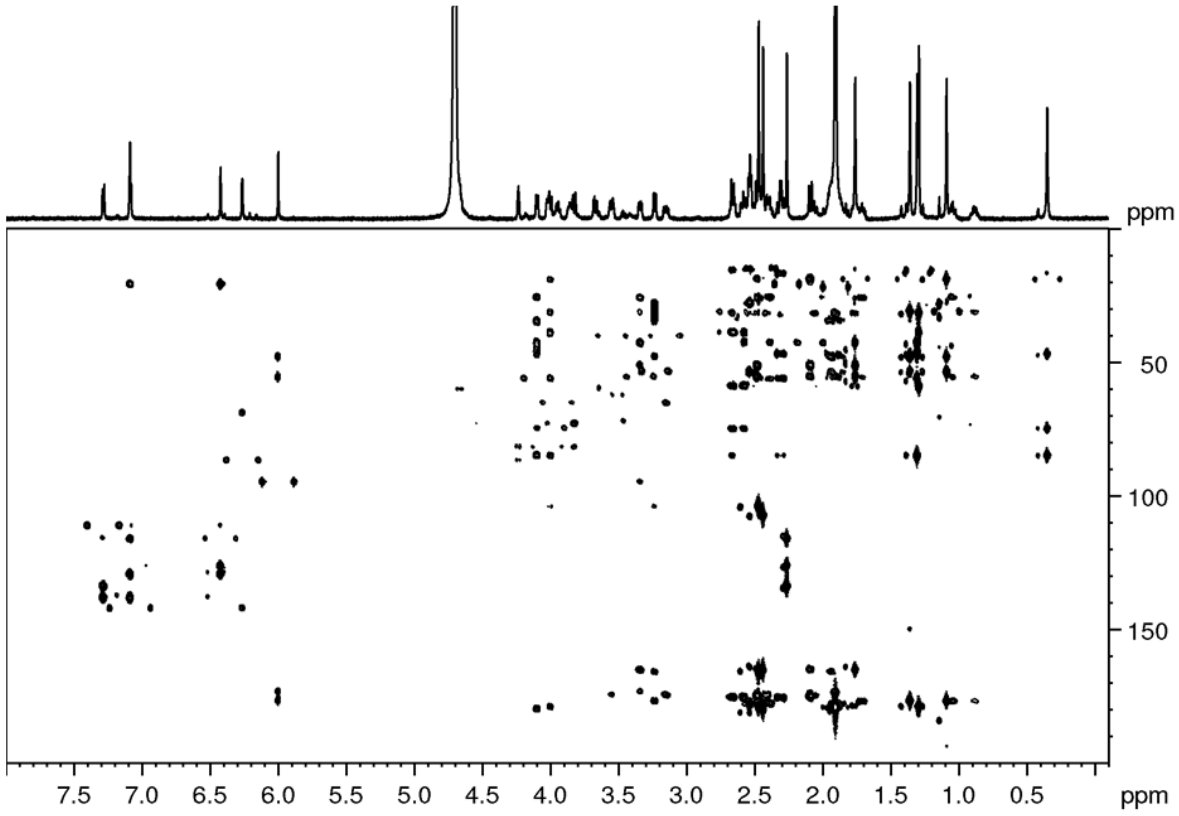

Fig. S20: ^{1}H-^{13}C HMBC spectrum of the 5-MeBza-NCba.

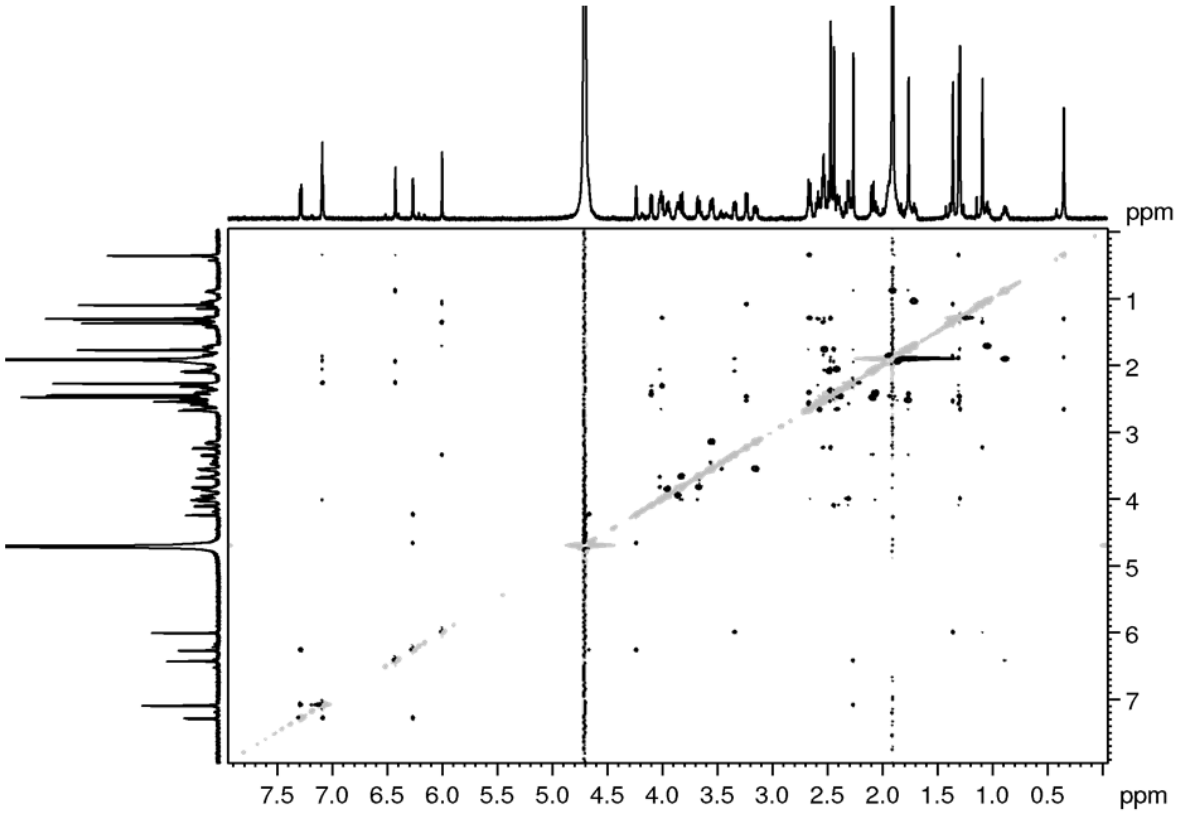

Fig. S21: ^{1}H-^{1}H ROESY spectrum of the 5-MeBza-NCba.

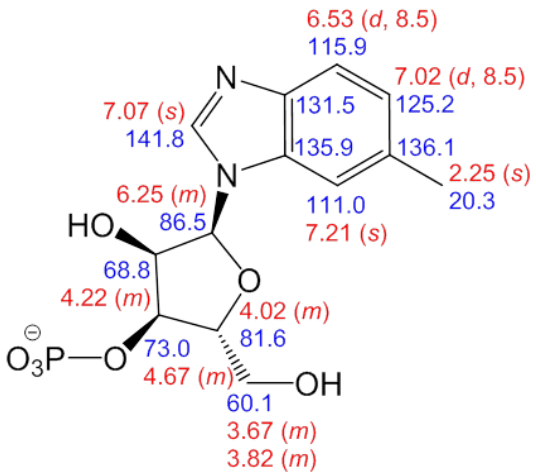

Fig. S22: Chemical shifts (δ_H red, δ_C blue) of the 6-MeBza-*N*-α-ribofuranosyl-fragment.

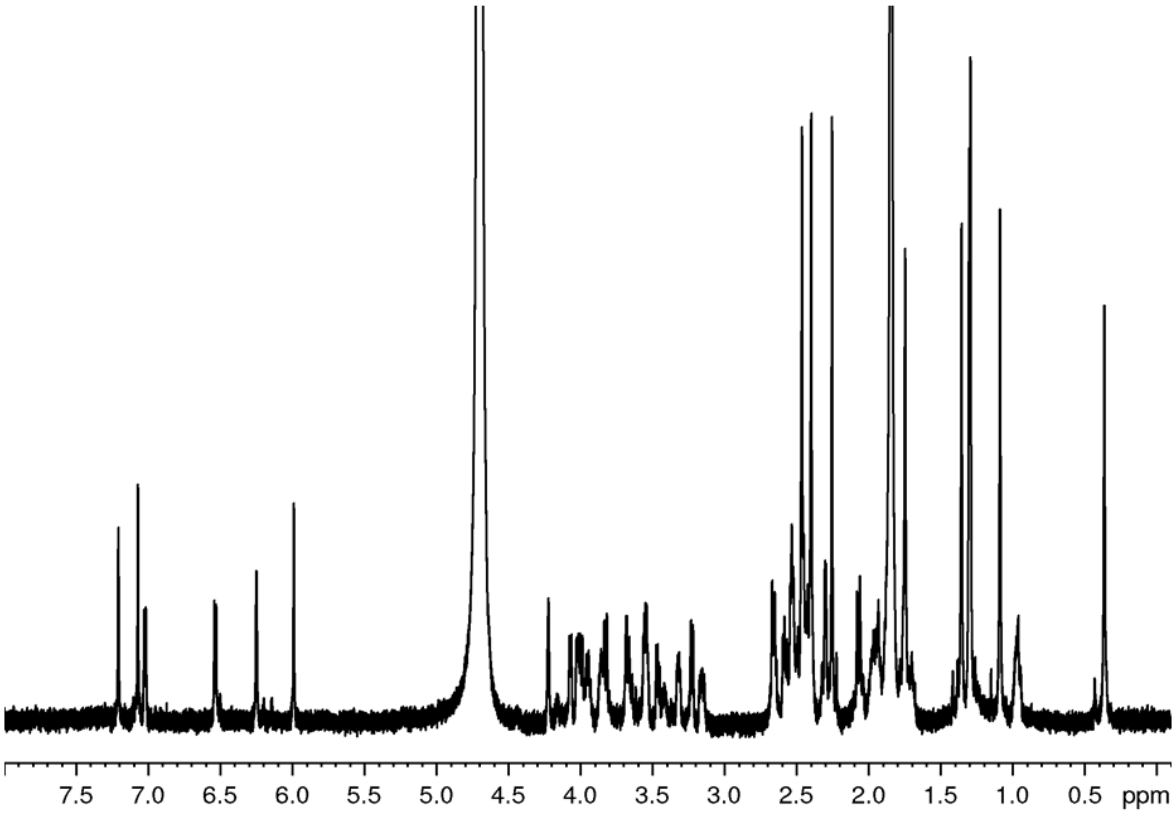

Fig. S23: ^{1}H-NMR spectrum of the 6-MeBza-NCba.

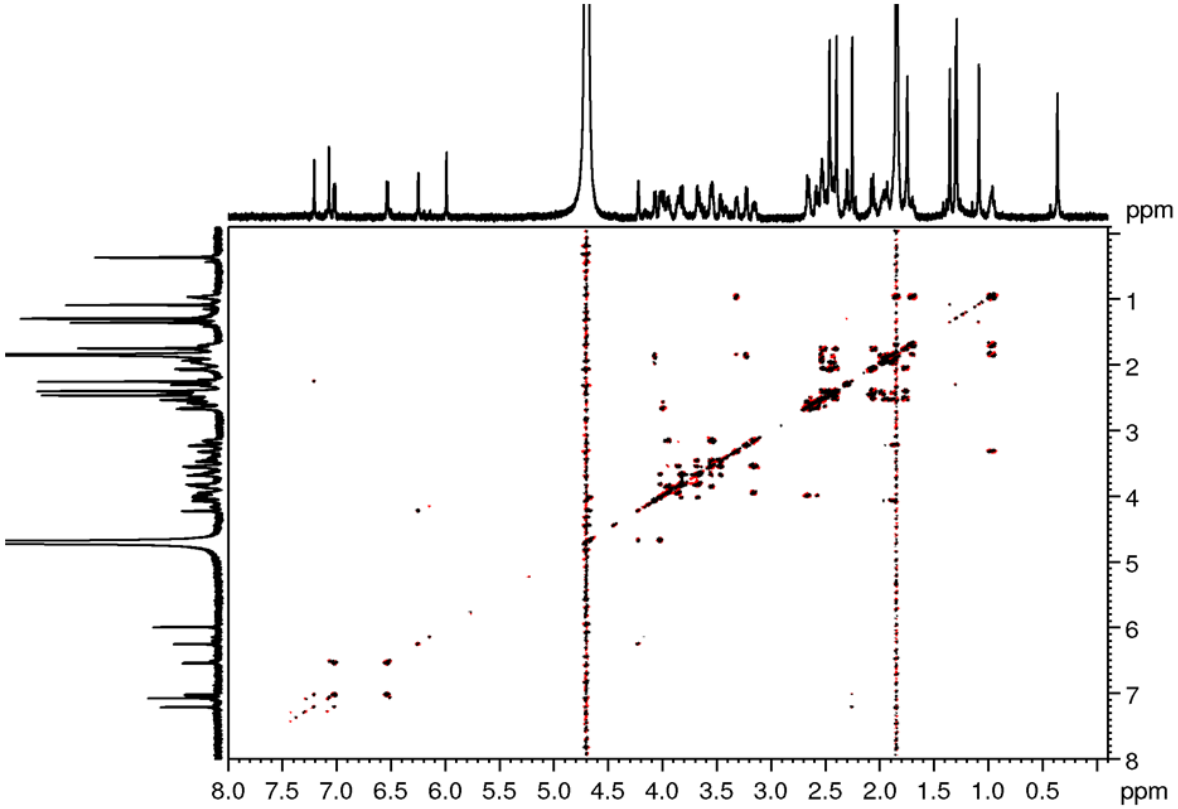

Fig. S24: ^{1}H-^{1}H-DQFCOSY spectrum of the 6-MeBza-NCba.

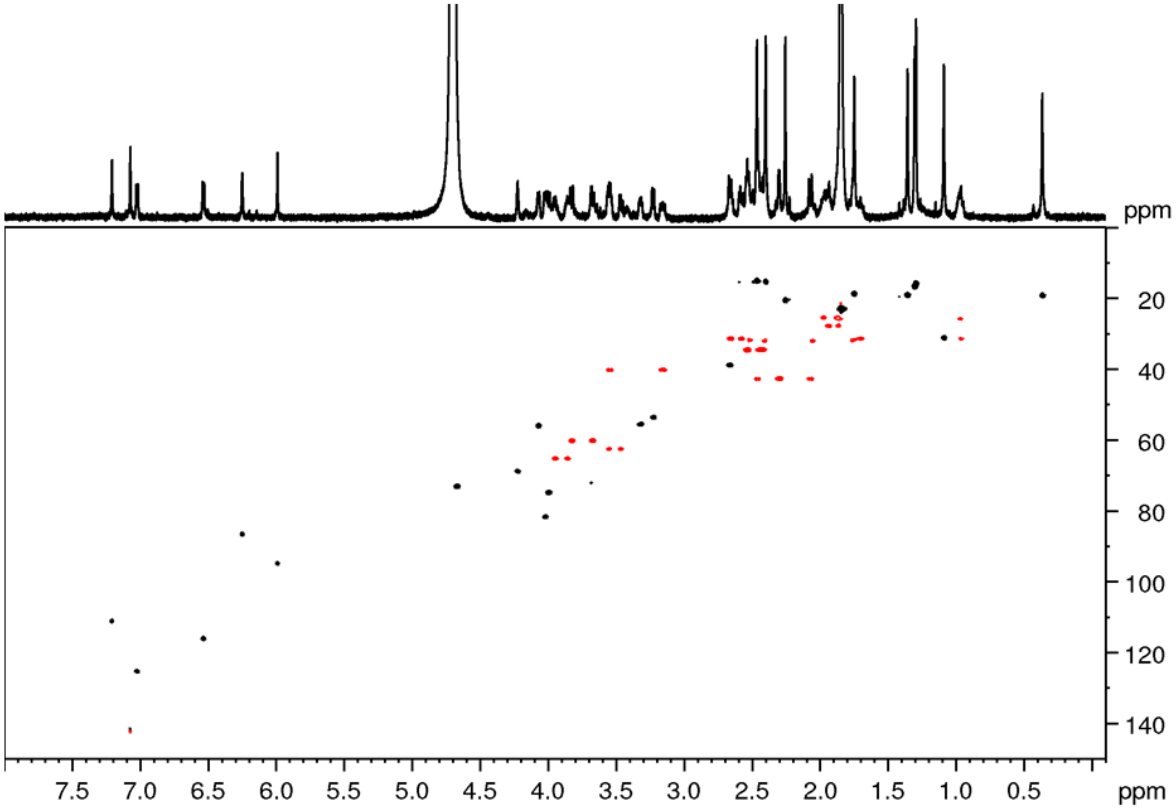

Fig. S25: ^{1}H-^{13}C HSQC spectrum of 6-MeBza-NCba.

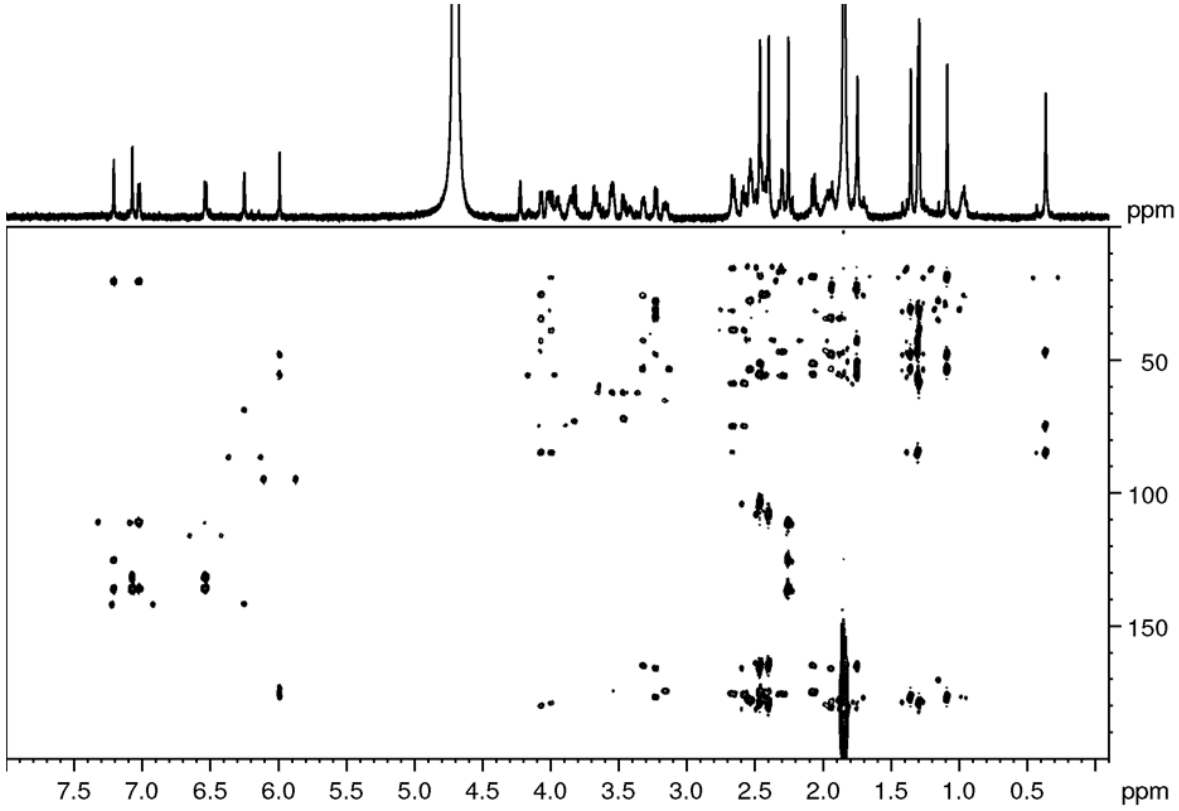

Fig. S26: ^{1}H-^{13}C HMBC spectrum of the 6-MeBza-NCba.

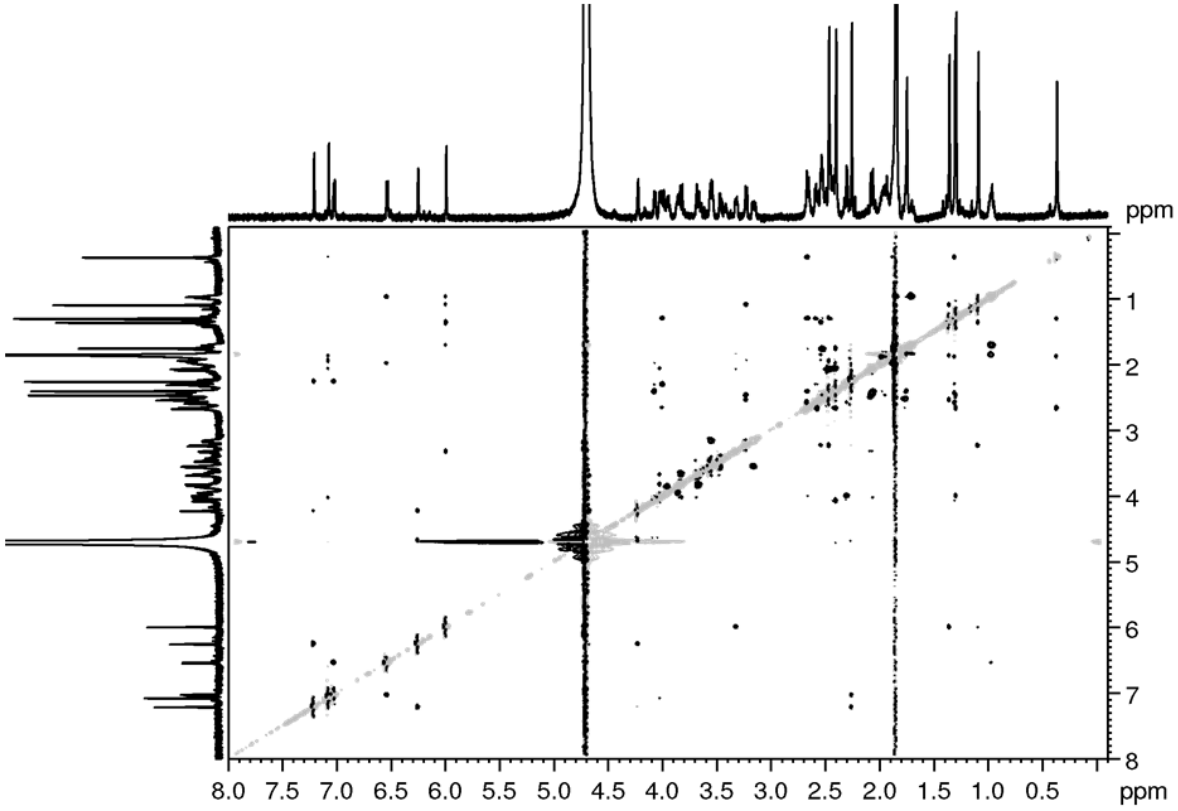

Fig. S27: ^{1}H-^{1}H ROESY spectrum of the 6-MeBza-NCba.

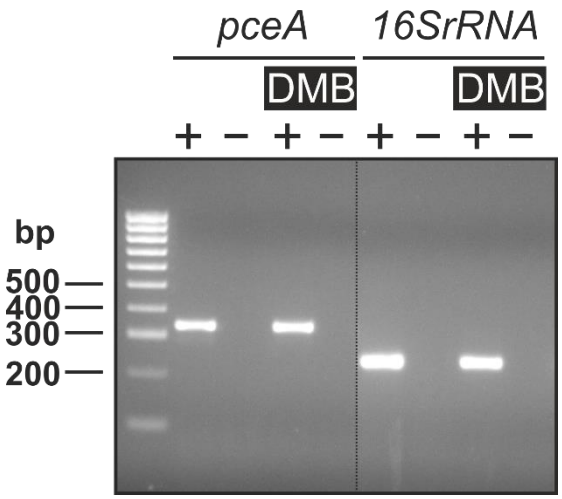

Fig. S28: Transcript level of the *pceA* gene in PCE-grown *S. multivorans* cultivated in the presence of DMB detected by reverse transcriptase PCR. The housekeeping gene *16SrRNA* was used as a control. (+) = with reverse transcription / (-) = without reverse transcription. Total RNA of *S. multivorans* (10^9 cells) was extracted with the RNeasy Mini Kit (Qiagen, Hilden, Germany) and applied to DNA digestion with DNase I (Roche, Mannheim, Germany) at 37°C for 1 h. RNA concentration was determined with a Qubit® 1.0 Fluorometers (Invitrogen, Darmstadt, Germany) and the Qubit® RNA BR Assay Kit (Thermo Fisher Scientific, Braunschweig, Germany). The reverse transcriptase (RT) reaction was performed with 1 µg total RNA using the OneStep RT-PCR Kit (Qiagen, Hilden, Germany) at 50°C for 1 h. The subsequent PCR was run as follows: initial activation (15 min, 95°C) and 20 cycles of polymerization (1 min 94°C; 30 sec 50°C; 1 min 72°C). The following primers were used: 5'-ACACATTAAAAAAT AAATAACTGTACTTGGGG-3' and 5'-TGAGTAAACGCTGTTCGTACTTCAGC-3' for *pceA* (fragment size: 339 bp) and 5'-GAGACACGGTCCAGACTCCTAC-3' and 5'-CTCGACTTGATTTCCAGCCTAC-3' for *16SrRNA* (fragment size: 255 bp).

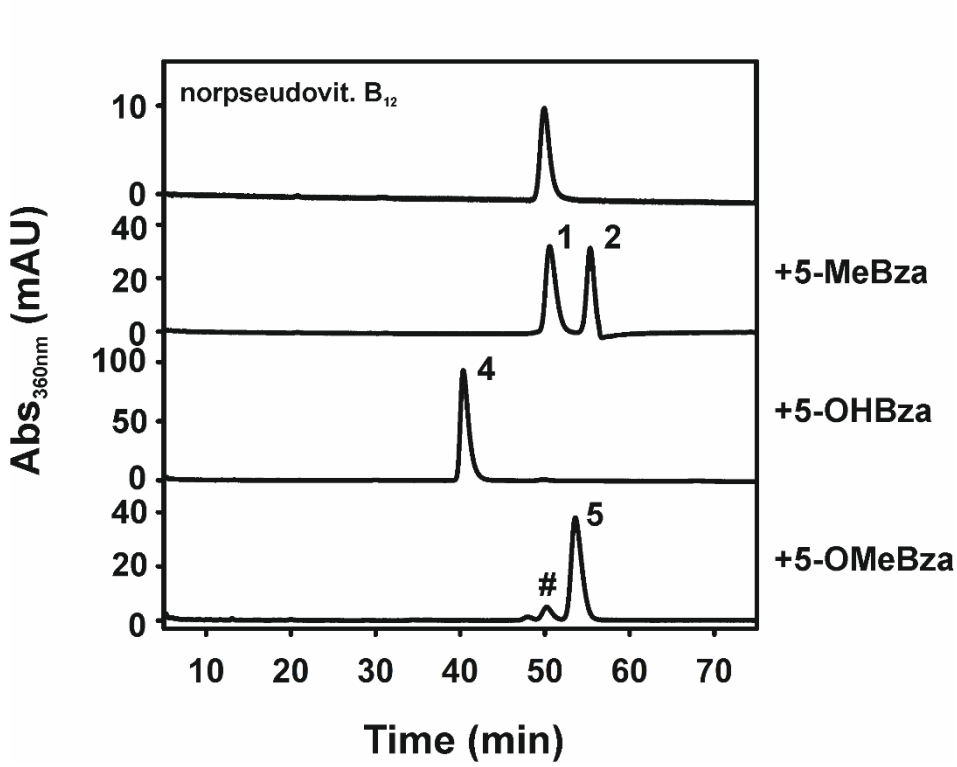

Fig. S29: HPLC-elution profiles of NCbas extracted from PceA purified from *S. multivorans* GD21 cultivated in the presence of either 5-MeBza, 5-OHBza, or 5-OMeBza. The first chromatogram shows a norpseudovitamin B_{12} standard. The designations of the signals refer to the NCbas identified in the respective cell types (see Fig. 1C). The specific activities of the purified PceA enzymes were as follows: + 5-MeBza, 687 nkat/mg; + 5-OHBza, 1056 nkat/mg; + 5-OMeBza, 1504 nkat/mg.

Tab. S2: Data collection and refinement statistics.

	PceA harboring **6-OHBza**-norcobamide	PceA harboring **5-OMeBza**-norcobamide
Data Collection	PETRA III P11	HZB-MX 14.1
Wavelength (Å)	0.9184	0.9184
Resolution range (Å)	36.1 - 1.59 (1.65 - 1.59)	39.8 - 1.60 (1.66 - 1.60)
Space group	*P* 4_1	*P* 4_1
Unit cell (Å, °)	73.6 73.6 184.7 90 90 90	73.6 73.6 185.3 90 90 90
Unique reflections	129361 (12146)	129127 (12767)
Multiplicity	13.2 (12.6)	13.8 (13.6)
Completeness (%)	99 (93)	100 (99)
I/sigma(I)	14.9 (1.9)	19.0 (1.7)
Wilson B-factor (Å^2)	19.0	20.2
R-merge	0.114 (0.931)	0.098 (1.57)
R-pim	0.033 (0.270)	0.027 (0.436)
$CC_{1/2}$	1.00 (0.80)	1.00 (0.62)
Refinement		
R-work	0.152 (0.257)	0.151 (0.260)
R-free	0.177 (0.285)	0.172 (0.289)
Number of non-H atoms	7945	7820
macromolecules	6838	6843
ligands	243	251
solvent	864	726
Protein residues	862	865
RMS(bonds, Å)	0.007	0.007
RMS(angles, °)	0.93	0.93
Ramachandran favored (%)	98	98
Ramachandran outliers (%)	0	0
Rotamer outliers (%)	0	0
Clashscore	2.7	2.4
Average B-factor (Å^2)	26.5	26.2
macromolecules	25.5	25.4
ligands	21.7	21.7
solvent	36.0	35.1