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Functionalization of Dimeric Cholestanopyrazines at the *quasi*-Benzylic Position

Zenon Łotowski, Eligiusz N. Dubis, and **Jacek W. Morzycki***

Institute of Chemistry, University of Bialystok, PL-15443 Bialystok, Poland

Summary. Two isomeric di-5α-cholestanopyrazines were brominated with *NBS/AIBN* at the *quasi*-benzylic positions (1α or 4α). The nucleophilic displacement of bromides with methanol afforded the corresponding methoxy derivatives. Both pyrazines gave mono- or di-N-oxides upon oxidation with *m*-chloroperoxybenzoic acid.

Keywords. Bromination; Cephalostatins; Natural products; Pyrazines; Steroids.

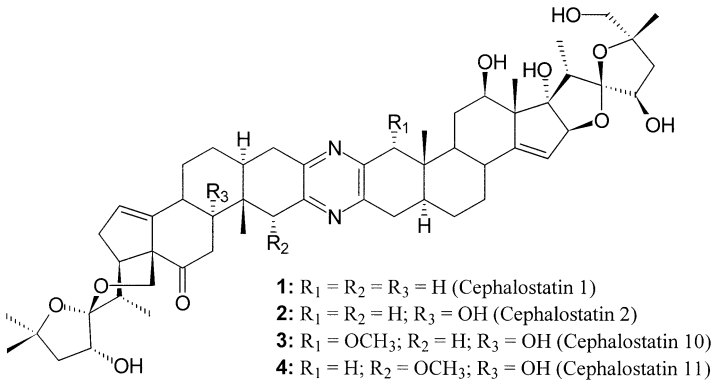
Funktionalisierung von dimeren Cholestanopyrazinen an der *quasi*-benzylischen Position

Zusammenfassung. Die isomeren di-5α-Cholestanopyrazine wurden mit *NBS/AIBN* an den *quasi*-benzylischen Positionen (1α oder 4α) bromiert. Nucleophile Substitution der Bromide mit Methanol führte zu den entsprechenden Methoxyderivaten. Die beiden Pyrazine gaben bei der Oxidation mit *m*-Chlorperbenzoesäure mono-und di-N-Oxide.

Introduction

Two families of dimeric steroid-pyrazine marine alkaloids, the cephalostatins [1] and ritterazines [2], have been recently discovered. These compounds exhibit an extraordinarily strong cytotoxic activity, superior to that of all standard chemotherapeutics, including taxol, adriamycin, cisplatin, 5-fluorouracil, and others [3]. Among the most active compounds [4] are cephalostatin 1 (**1**), cephalostatin 2 (hydroxylated at C-9′; **2**), and its 1α-methoxy (**3**) and 1′α-methoxy (**4**) derivatives (cephalostatins 10 and 11, respectively). Synthetic analogs of cephalostatins functionalized at C-1 have also proved strongly cytotoxic [5]. Therefore an attempt to introduce a functional group into this position in a system related to cephalostatin was undertaken. As model compounds for this study di-5α-cholestanopyrazines were chosen.

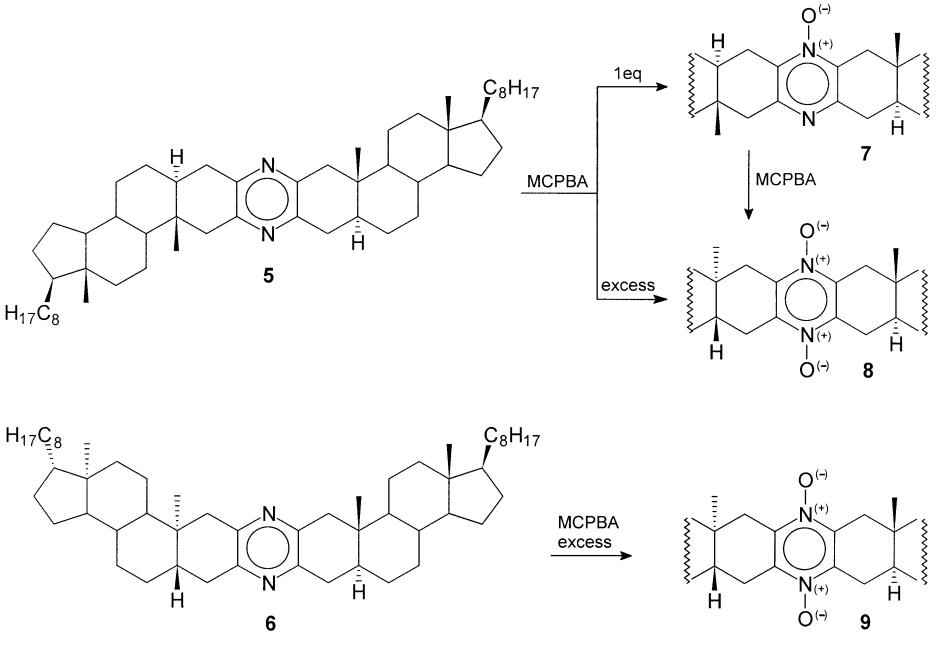
* Corresponding author



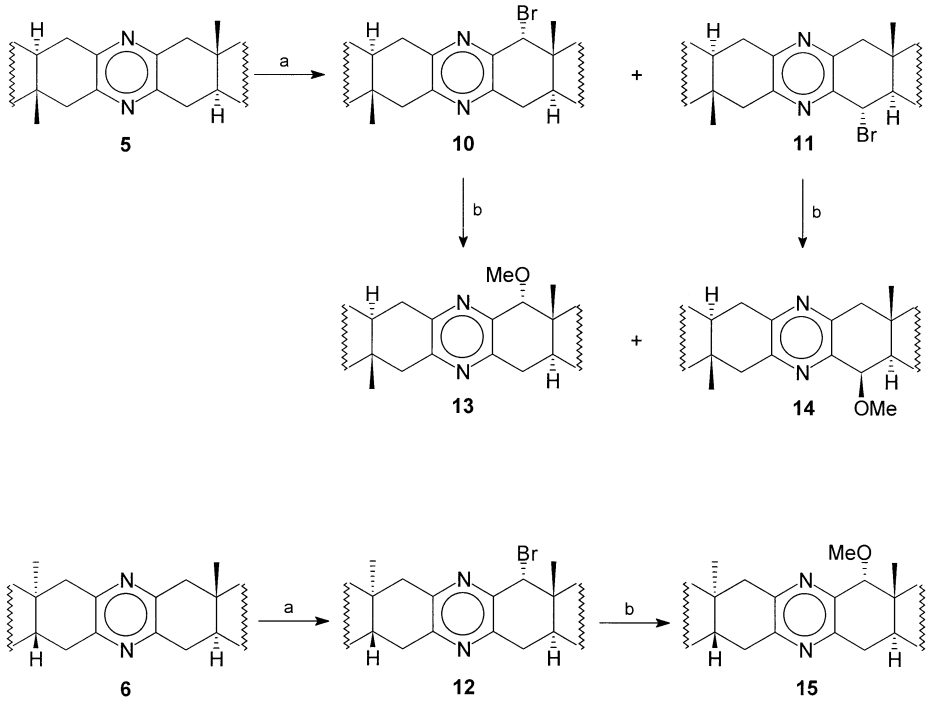
Scheme 1

Results and Discussion

In a previous paper [6] we have described a simple procedure for the pyrazine ring construction involving reaction of the steroidal 2α-bromo-3-ketone with ammonia and leading to an easily separable mixture of the *trans* and *cis* pyrazine isomers. Now we report a method to introduce a methoxy group into the *quasi*-benzylic 1α-position of the dimeric steroid-pyrazines. This study was performed on the model systems of the cholestane dimers **5** and **6** [6, 7] as well as their N-oxides. It is well known that N-oxides deriving from substituted pyridine systems treated with acetic anhydride undergo rearrangement to pyridines bearing an oxygen substituent in the side chain [8]. It was expected that a similar reaction might occur in the case of pyrazine N-oxides. Both isomers **5** and **6** are C_2-symmetrical like their di-N-oxides



Scheme 2



Scheme 3

in contrast to the mono-N-oxide of the *trans* isomer **5** which is C_1-symmetrical. The *trans* isomer **5** was easily transformed to its mono- and di-N-oxides **7** and **8** with an equivalent amount or an excess of *m-CPBA*, respectively. The *cis* isomer gave two mono-N-oxides both of which are C_2-symmetrical. However, they were unseparable by means of chromatography, and for this reason the mixture was converted to the di-N-oxide **9** with an excess of *m-CPBA*. The differences in the properties of their N-oxides permitted to distinguish between the *trans* and the *cis* isomers; the NMR spectra of the pyrazines are nearly identical.

Compounds **5–9** were oxidized under various conditions (*e.g.* CrO_3/Py, CrO_3/HOAc, $CrO_3/Ac_2O/H_2SO_4$, $KMnO_4/CuSO_4$, SeO_2/*t*-BuOOH, $Pb(OAc)_4$, *etc.*), but all these reactions failed to produce clean oxidation at the *quasi*-benzylic position. Attempts to introduce an acetoxyl group into this position by treatment of N-oxides with acetic anhydride [8] were also unsuccessful. The best results were obtained for a radical bromination of **5** and **6** with *NBS* (1 eq) in the presence of *AIBN* (the reactions of N-oxides were not as clean). The bromination took place at the desired position fairly selectively. In the case of the *trans* isomer **5** a mixture of the 1α-bromo and 4α-bromo derivatives **10** and **11** in a ratio of 2:1 was obtained in 44% yield. From this mixture **10** could be easily isolated by crystallization. Monobromination of the *cis* isomer **6** afforded selectively the corresponding 1α-bromo derivative **12** in 28% yield. In both cases bromine attacked the dimeric steroid-pyrazine from its less hindered α-side. The observed regioselectivity is

Table 1. Calculated steric energies of methoxy-di-5α-cholestanopyrazines in the *trans* and *cis* series (HyperChem^{TM}, Release 3 from Hypercube, Inc.; minimizations employed the MM+ force field and the *Polak-Ribiere* (conjugate gradient) algorithm)

	Compound	Steric energy/kJ · mol^{-1}
trans series:	1α-OMe (**13**)	25.4
	1β-OMe	26.7
	4α-OMe	25.9
	4β-OMe (**14**)	24.2
cis series:	1α-OMe (**15**)	26.7
	1β-OMe	27.6

probably the result of the lower energy of the carbon radical at C-1 compared with that at C-4. The bromides **10**–**12** were subjected to methanolysis catalyzed by *p-Ts*OH. The reaction conditions favor the S_N1 mechanism proceeding *via* the relatively stable *quasi*-benzylic carbocation. The product formation is then under thermodynamic control. The relative stability of the methoxy derivatives (*i.e.* their steric energies) is shown in Table 1.

In compliance with the above considerations methanolysis of 1α-bromo compounds **10** and **12** proceeded with retention of configuration, whereas during the reaction of the 4α-bromo compound **11** inversion at C-4 was observed. In all cases the more stable methoxy derivatives were formed. The configuration of the methoxy groups in **13**–**15** was established on the basis of their ^{1}H NMR spectra. The signal of the *quasi*-equatorial proton at $\delta = 4.08$ ppm ($J = 4.2$ Hz) proved the β orientation of the methoxy group at C-4 in **14**. The C-1 protons in **13** and **15** are not coupled, and therefore the shape of their signals (singlets at 3.93 and 3.94 ppm) cannot prove the methoxy group configurations. However, an NOE enhancement (1.2% and 1.8% for **13** and **15**) of these signals was observed upon irradiation of the angular C-19 methyl protons, proving the 1α-configurations of the methoxy group in these compounds. Displacement of bromine by methoxyl was faster at C-4 than at C-1. Kinetic separation of a mixture of 1α- and 4α-bromides **10** and **11** was therefore possible by transforming it into the easily separable mixture of **10** and the 4β-methoxy compound **14**.

Experimental

Melting points were determined on a Kofler apparatus of the Boëtius type and are uncorrected. NMR spectra were taken with a Bruker AC 200F spectrometer (200 MHz) using $CDCl_3$ solutions with *TMS* as an internal standard. Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer for chloroform solutions unless stated otherwise. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J. T. Baker). Thin-layer chromatograms were developed on aluminum TLC sheets precoated with silica gel F_{254} and visualized with 50% sulfuric acid after heating. All solvents were dried and freshly distilled prior to use. Educts **5** and **6** were prepared according to Ref. [6].

Di(cholestano[2,3-b:2′,3′-e])pyrazine mono-N-oxide (**7**; $C_{54}H_{88}N_2O$)

A solution of 50 mg (0.15 mmol) of *m-CPBA* (50%, Aldrich) in 1 cm^3 $CHCl_3$ was prepared, dried (anh. $MgSO_4$), and added portionwise to a stirred reaction mixture consisting of 100 mg **5** (0.13 mmol) dissolved in 10 cm^3 $CHCl_3$ and 300 mg anh. $NaHCO_3$. The addition was completed within 30 min, and stirring was continued for further 5 min. The reaction mixture was placed on top of a silica gel column. Elution with benzene-chloroform (65:35) gave the pure pyrazine mono-N-oxide (**7**; 69 mg; 68%).

M.p.: 315–323°C (hexane-CH_2Cl_2); IR ($CHCl_3$): $\nu = 1339$, 1132, 1097 cm^{-1}; ^1H NMR ($CDCl_3$, δ, 200 MHz): 2.8–3.3 (m, 4H, *quasi*-benzylic protons), 2.4–2.8 (m, 4H, *quasi*-benzylic protons), 0.79 and 0.775 (2×s, 2×3H, 19-H and 19′-H), 0.694 and 0.685 (2×s, 2×3H, 18-H and 18′-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 151.8 (C), 151.7 (C), 139.8 (C), 139.2 (C) ppm (heteroaromatic carbons); MS: *m/z* (%) = 781 (MH^+, 20), 763 (14), 154 (100).

Di(cholestano[2,3-b:2′,3′-e])pyrazine bis-N-oxide (**8**)

A solution of 100 mg (0.3 mmol) of *m-CPBA* (50%, Aldrich) in 2 cm^3 $CHCl_3$ was prepared, dried (anh. $MgSO_4$), and added portionwise to a stirred reaction mixture consisting of 100 mg **5** (0.13 mmol) dissolved in 10 cm^3 $CHCl_3$ and 300 mg anh. $NaHCO_3$. The addition was completed within 45 min, and stirring was continued overnight. The reaction mixture was placed on top of a silica gel column. Elution with benzene-chloroform (1:1) gave the pure pyrazine di-N-oxide (**8**; 78 mg, 75%).

M.p: 312°C (dec.) (hexane-CH_2Cl_2); its analytical and spectroscopic properties were identical with those described in Ref. [7].

Di(cholestano[2,3-b:3′,2′-e])pyrazine bis-N-oxide (**9**; $C_{54}H_{88}N_2O_2$)

The pyrazine di-N-oxide **9** was obtained from **6** in a way similar to the preparation of **8** in 69% yield.

M.p.: 267–270°C (hexane-CH_2Cl_2); IR ($CHCl_3$): $\nu = 1336$, 1155, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, δ, 200 MHz): 3.23 (d, $J = 18.6$ Hz, 2H), 3.04 (dd, $J = 19.4$ Hz, 4.5 Hz, 2H), 2.44 (m, 2H) and 2.28 (brd, $J = 19.4$ Hz, 2H), *quasi*-benzylic protons, 0.75 (s, 6H, 19-H), 0.68 (s, 6H, 18-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 142.9 (2×C), 141.9 (2×C), 56.2 (4×CH), 53.5 (2×CH), 42.4 (2×C), 40.1 (2×CH), 39.7 (2×CH_2), 39.5 (2×CH_2), 38.1 (2×CH_2), 36.1 (2×CH_2), 35.7 (2×CH), 35.3 (2×CH), 34.5 (2×C), 31.2 (2×CH_2), 28.3 (2×CH_2), 28.2 (2×CH_2), 28.0 (2×CH), 27.9 (2×CH_2), 24.1 (2×CH_2), 23.8 (2×CH_2), 22.8 (2×CH_3), 22.5 (2×CH_3), 21.3 (2×CH_2), 18.6 (2×CH_3), 12.2 (2×CH_3), 12.0 (2×CH_3) ppm; MS: *m/z* (%) = 797 (MH^+, 100), 781(29), 154 (26).

1α-Bromo-di(cholestano[2,3-b:2′,3′-e])pyrazine (**10**; $C_{54}H_{87}BrN_2$) and
4α-bromo-di(cholestano-[2,3-b:2′,3′-e])pyrazine (**11**; $C_{54}H_{87}BrN_2$)

100 mg **5** (0.13 mmol), 24 mg *NBS* (0.13 mmol), and 18 mg *AIBN* (0.11 mmol) were dissolved in 15 cm^3 anh. CCl_4, and the reaction mixture was heated under reflux for 1 h. The solvent was removed *in vacuo*, and the residue was subjected to silica gel column chromatography. Elution with benzene-hexane (6:4) afforded 48 mg (44%) of a mixture of bromides **10** and **11** in a ratio of 2:1. Pure 1α-bromo product **10** was obtained by crystallization of the mixture from hexane-CH_2Cl_2.

M.p.: 314–318°C (dec.); IR ($CHCl_3$): $\nu = 1398$, 1384 cm^{-1}; ^1H NMR ($CDCl_3$, δ, 200 MHz): 5.17 (s, 1H, 1β-H), 2.8–3.05 (m, 3H, *quasi*-benzylic protons), 2.4–2.7 (m, 3H, *quasi*-benzylic protons), 0.89 (s, overlapped by the side chain signals, 19-H), 0.76 (s, 3H, 19′-H), 0.69 (s, 6H, 18-H and 18′-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 151.9 (C), 149.4 (C), 148.2 (C) and 147.6 (C) (heteroaromatic carbons) 64.1 (CHBr) ppm; MS: *m/z* (%) = 843 and 845 (MH^+, 23), 763 (20), 154 (100); HRMS:

calcd. for $C_{54}H_{88}N_2{}^{79}Br$ (MH^+): 843.6131, found: 843.6146; calcd. for $C_{54}H_{88}N_2{}^{81}Br$. 845.6110, found: 845.6126.

The 4α-bromo product **11** could not be obtained in its pure form from the mother liquor containing up to 70% of **11** (a signal of 4β-H in its ^{1}H NMR spectrum appeared at $\delta = 4.98$ ppm(d, $J = 10.35$ Hz)). A mixture of both bromo products **10** and **11** was used in the further transformations.

1α-Bromo-di(cholestano[2,3-b:3′,2′-e])pyrazine (**12**; $C_{54}H_{87}BrN_2$)

115 mg **6** (0.15 mmol), 27 mg *NBS* (0.15 mmol), and 20 mg *AIBN* (0.12 mmol) were dissolved in 17 cm^3 anh. CCl_4, and the reaction mixture was heated under reflux for 1 h. The solvent was removed *in vacuo*, and the residue was subjected to silica gel column chromatography. Elution with benzene yielded 36 mg (28%) of pure, amorphous 1α-bromo product **12**.

IR ($CHCl_3$): $\nu = 1398$, 1383, cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 5.15 (s, 1H, 1β-H), 2.3–3.1 (m, 6H, *quasi*-benzylic protons), 0.88 (s, 3H, 19-H), 0.84 (s, 3H, 19′-H), 0.69 (s, 6H, 18-H and 18′-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 151.3 (C), 149.9 (C), 148.3 (C) and 147.6 (C) (heteroaromatic carbons), 63.9 (CHBr) ppm; MS: *m/z* (%) = 762 (M^+-HBr, 100), 747 (34), 502 (36), 487 (27).

1α-Methoxy-di(cholestano[2,3-b:2′,3′-e])pyrazine (**13**; $C_{55}H_{90}N_2O$) and
4β-methoxy-di(cholestano-[2,3-b:2′,3′-e])pyrazine (**14**; $C_{55}H_{90}N_2O$)

A mixture of 100 mg of bromides **10** and **11** (0.12 mmol) obtained according to the procedure described above was dissolved in 1 cm^3 dioxane and 1 cm^3 $CHCl_3$. Then 20 cm^3 methanol were added, and the reaction mixture was heated under reflux in the presence of a catalytic amount of *p-Ts*OH for 6 h. The solvents were removed *in vacuo* and the products were separated by silica gel column chromatography. Elution with benzene-hexane (1:1) afforded 48 mg of unreacted bromide **10**. Further elution with benzene-hexane (7:3) yielded consecutively the methoxy products **14** (28 mg) and **13** (16 mg).

13: M.p.: 281–284°C (hexane); IR ($CHCl_3$): $\nu = 1398$, 1078, 909 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 3.93 (s, 1H, 1β-H), 3.41 (s, 3H, CH_3O), 2.78–3.04 (m, 3H, *quasi*-benzylic protons), 2.28–2.70 (m, 3H, *quasi*-benzylic protons), 0.82 (s, 3H, 19′-H), 0.691, 0.685 and 0.673 (3×s, 9H, 18-H, 18′-H and 19-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 151.1 (C), 149.5 (C), 147.8 (C) and 147.6 (C) (heteroaromatic carbons), 83.4 (CHOMe) ppm; MS: *m/z* (%) = 794 (M^+, 1), 779 (1), 764 (100), 749 (47).

14: M.p.: 306–309°C (MeOH-CH_2Cl_2); IR ($CHCl_3$): $\nu = 1401$, 1113, 1077 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 4.08 (d, $J = 4.2$ Hz, 1H, 4α-H), 3.66 (s, 3H, CH_3O), 2.99 (d, $J = 17.4$ Hz, 2H, *quasi*-benzylic protons), 2.36–2.88 (m, 4H, *quasi*-benzylic protons), 1.00 and 0.83 (2×s, 2×3H, 19-H and 19′-H), 0.70 and 0.68 (2×s, 2×3H, 18-H and 18′-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 150.7 (C), 149.6 (C), 148.4 (C) and 148.2 (C) (heteroaromatic carbons), 81.8 (CHOMe) ppm; MS: *m/z* (%) = 794 (M^+, 2), 779 (2), 764 (100), 749 (6).

In a separate experiment it was checked that in order to complete the methanolysis, the bromide **10** must be refluxed in methanol/*p-Ts*OH for 6 days.

1α-Methoxy-di(cholestano[2,3-b:3′,2′-e])pyrazine (**15**; $C_{55}H_{90}N_2O$)

To a solution of 20 mg bromide **12** (0.02 mmol) in dioxane and $CHCl_3$ (1 cm^3 each), 10 cm^3 methanol were added, and the reaction mixture was heated under reflux with a catalytic amount of *p-Ts*OH for 6 days. The solvents were then removed *in vacuo* and the crude product was purified by silica gel column chromatography. Elution with benzene-chloroform (1:1) afforded 11 mg (58%) of the 1α-methoxy compound **15**.

M.p.: 205–208°C (MeOH-CH_2Cl_2); IR ($CHCl_3$): $\nu = 1400$, 1383, 1087, 1078 cm^{-1}; ^1H NMR ($CDCl_3$, δ, 200 MHz): 3.94 (s, 1H, 1β-H), 3.41 (s, 3H, CH_3O), 2.72–3.04 (m, 3H, *quasi*-benzylic protons), 2.38–2.70 (m, 3H, *quasi*-benzylic protons), 0.81 (s, 3H, 19′-H), 0.691, 0.681 and 0.663 (3×s, 9H, 18-H, 18′-H and 19-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 150.5 (C), 149.3 (C), 148.4 (C) and 147.7 (C) (heteroaromatic carbons), 83.3 (CHOMe) ppm; MS: m/z (%) = 794 (M^+, 1), 779 (1), 764 (100), 749 (31).

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