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INSTITUT FÜR ORGANISCHE CHEMIE

DER UNIVERSITÄT HANNOVER

Univ.-Prof.Dr.Dr.h.c.E.Winterfeldt

D - 30167 H A N N O V E R

Schneiderberg 1b

Telefon (0511) 762-4649/4613 (dienstl.)

Fax (0511) 762-3011 (dienstl.)

Telefon (0511) 9734810 (privat)

Fax (0511) 9734327 (privat)

WINTERFELDT@mbox.OCI.UNI-HANNOVER.DE

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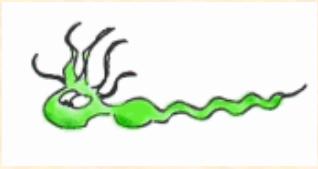
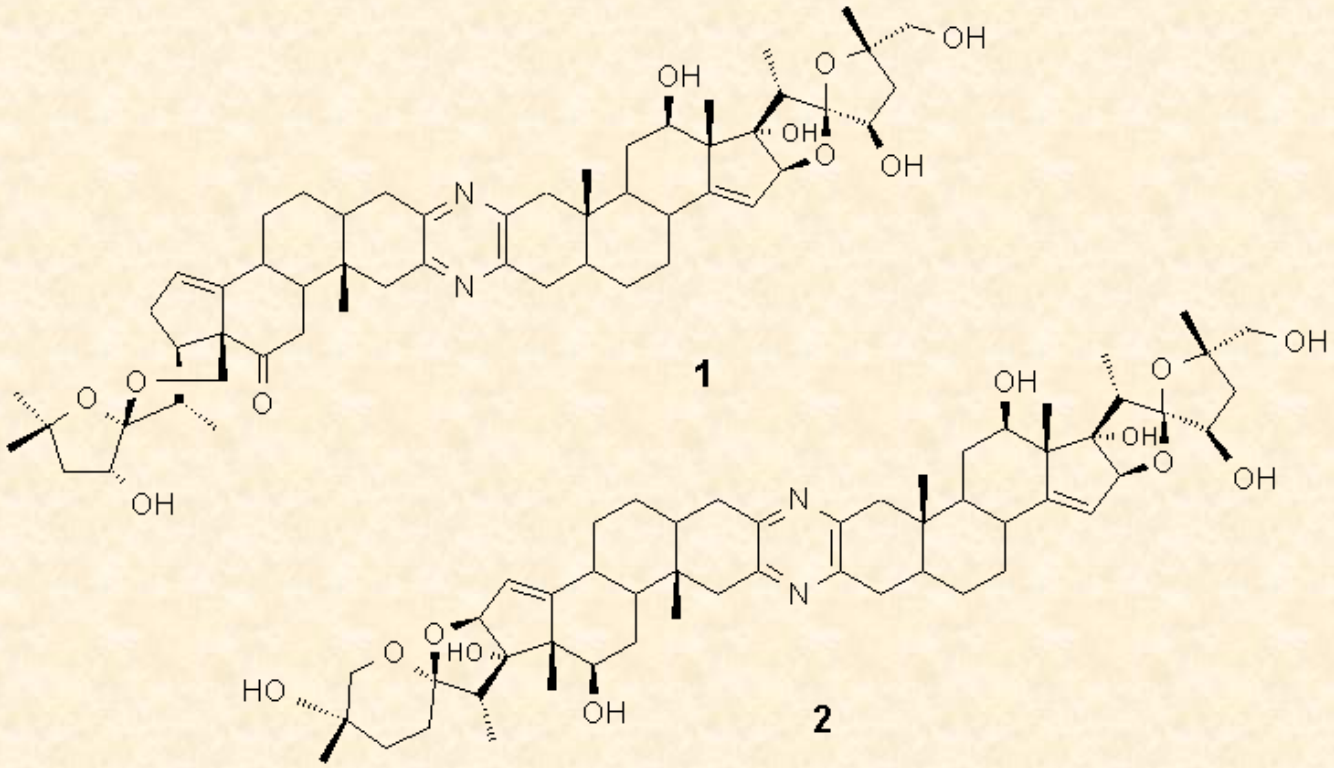
Steroidal pyrazines, synthetic advances and biological activity



Michael Drögemüller, Timo Flessner, Rolf Jautelat, Mansour Nawasreh, Ulrich Scholz and Ekkehard Winterfeldt*

Hyperlinks on compound names will lead you to additional information, such as experimental data, three dimensional structures in the ENT Protein Database format, or additional schemes.

After the novel and unusual structure of the cephalostatins (scheme 1) had been elucidated [1] and the limited availability of these powerful cytotoxins had been realized[2], synthetic efforts aiming at compounds of this type became known from various places in the world.

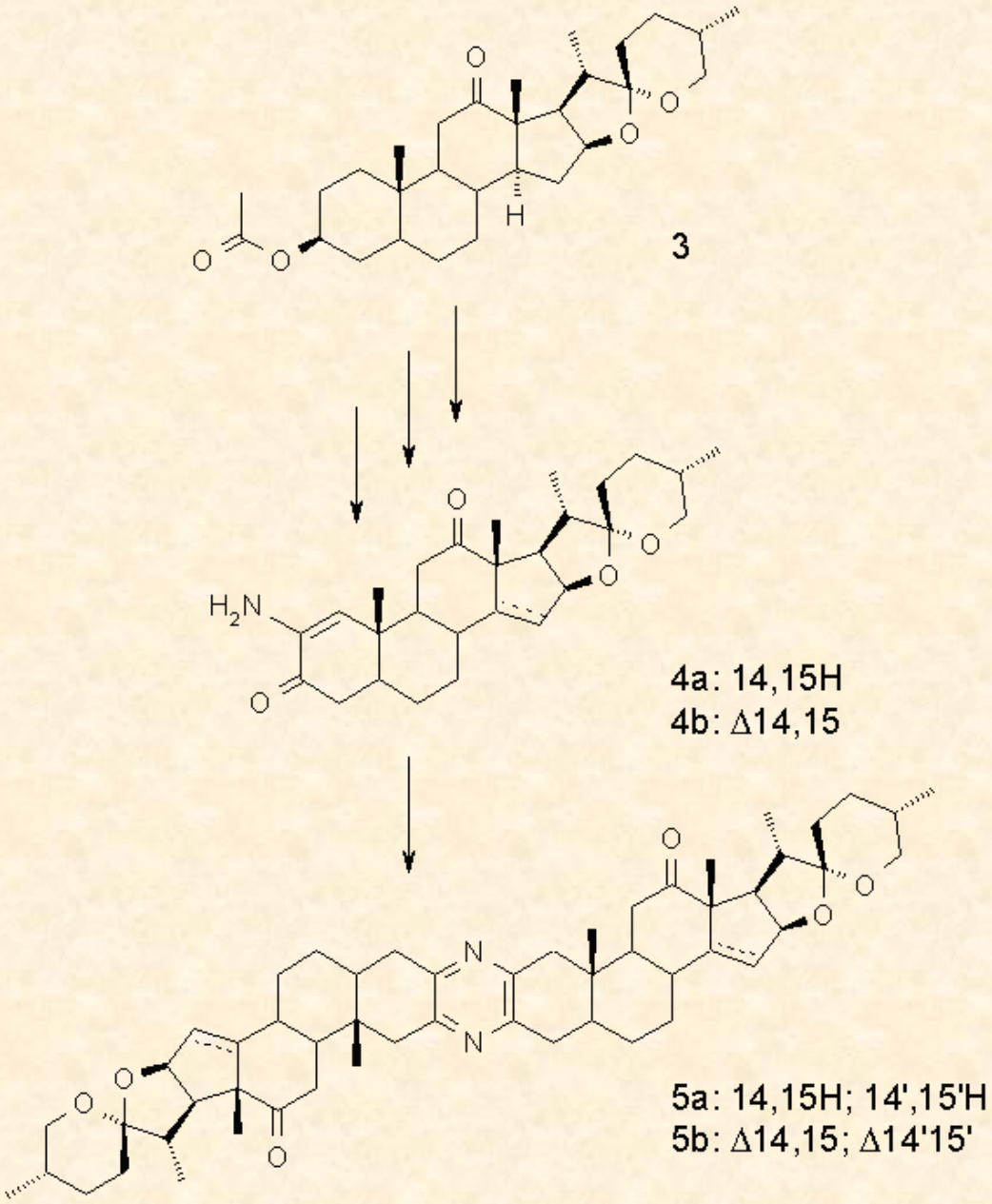


scheme 1: Cephalostatin I **(1)** and Cephalostatin VII (2)

Very important contributions to this field were reported from Clayton Heathcock's laboratory [3] and additionally one has to particularly mention Peter Fuchs and his collaborators who, after developing elegant routes to the corresponding steroid building blocks, quite recently published the first total synthesis of cephalostatin I [4].

With the investigations in our laboratory we mainly had the intention to elaborate short routes to biologically active cephalostatin analogues from easily available steroidal natural compounds such as hecogenin and to determine the essential substructures for the extraordinary high cytotoxicity of these compounds.

Since the D 14,15 double bond seemed to be characteristic for the biological activity of this group of tumor inhibitors, we addressed this aspect first and prepared the symmetric diketones **5a** and **5b** [5,6].



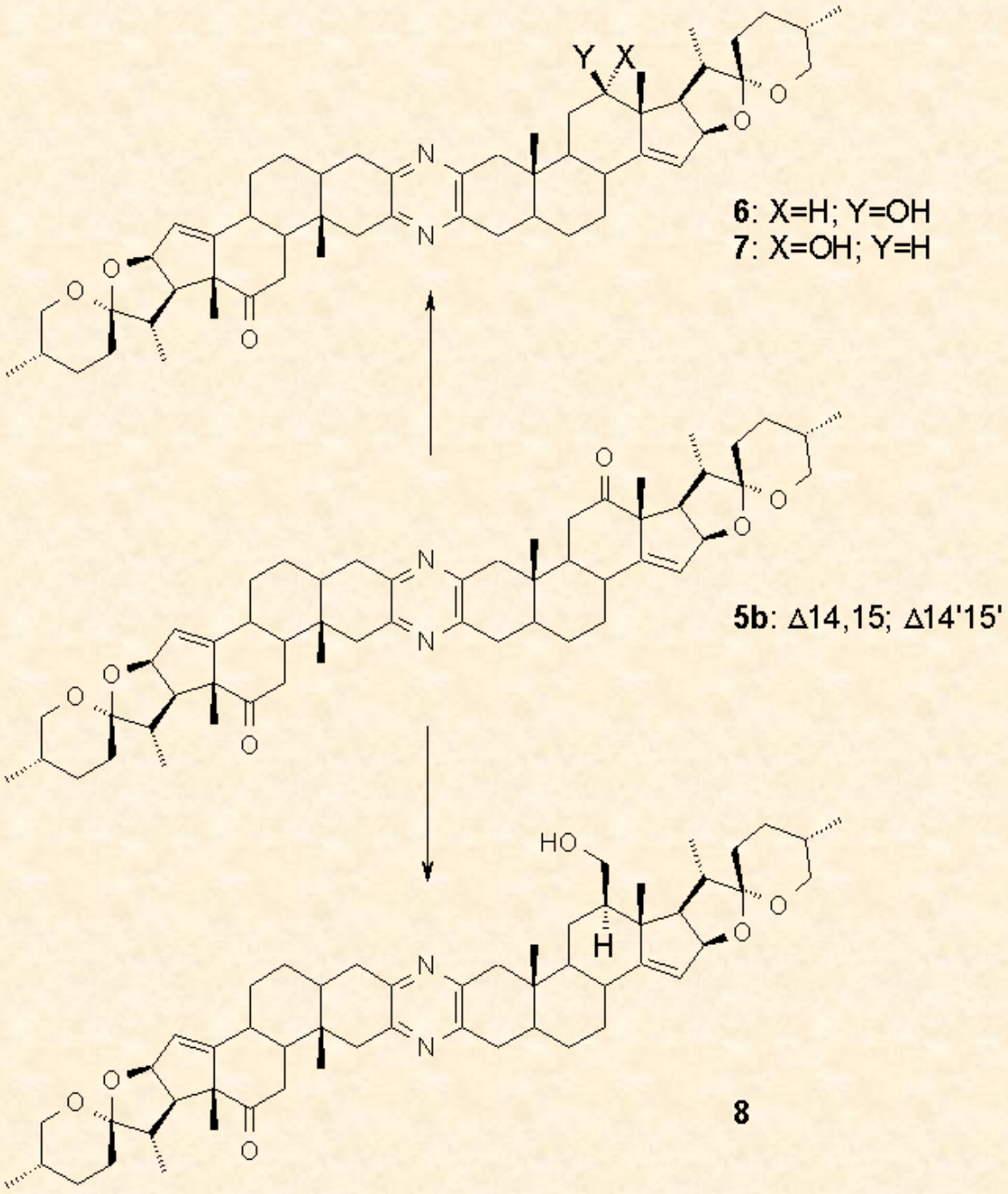
scheme 2: Synthesis of symmetrical analogues

On evaluation of **5a** very weak biological activity went along with extremely low solubility in organic solvents, while the D 14,15 derivative **5b** did not only show much better solubility but also affected a considerably higher number of cell-lines. This remarkably improved solubility was noticed with all the various D 14,15 compounds that were prepared during this project [7] and we thus assume that this property is caused by the chiral curvature that is induced in these compounds by this very double bond. Picture 1 shows the two sp^2 centers in the five membered ring to cause 'a bending down of the wing-tips' of this otherwise planar molecule.

picture 1: first biologically active analogues

Various observations in our laboratory [8] together with very interesting X-ray data published by *J.M.Lehn* [9] indicate very clearly that molecules with a well defined chiral curvature that are only available as one single enantiomer, are characterized by a less dense packing in the crystal-lattice. This is explained by the absence of the corresponding complementing enantiomer which also accounts for the higher solubility of the single stereoisomer. To check this theory presented here one would need the non natural enantiomer of **5a** which is unfortunately not available.

The biological activity increased even further, when the 12-keto group was either selectively reduced to the 12b -hydroxyketone **6** ($NaBH_4$) or the 12a -hydroxyketone **7** (L-selectride), or homologated to hydroxyketone **8,** which can be obtained in a selective *Wittig*-methylenation-hydroboration sequence[10], (scheme 3). Since all these compounds showed remarkable cytotoxicity, we had to draw the conclusion that the D 14,15 double bond together with the central pyrazine ring and molecular dissymmetry are indispensable essentials for tumor inibiting properties of this group of natural compounds.



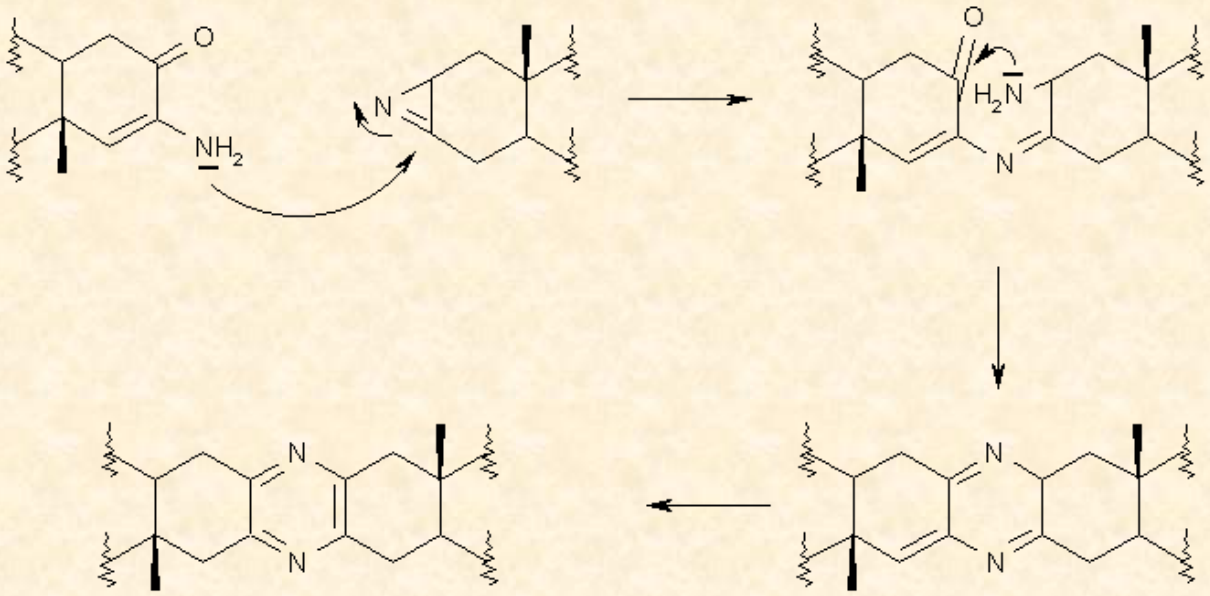
scheme 3: unsymmetrical analogues

As an inspection of the molecular structures of all the cephalostatins known today proves all of them to be non-symmetric too, it was evident that one would certainly need a general synthetic approach to non-symmetric pyrazines.

Although techniques for the preparation of pyrazines were of course known, they either show no substrate specifity as for instance the preparation by reaction of two a -aminoketones, or they did not favour the formation of one regioisomer as for instance the condensation of a -diamines with a -diketones.

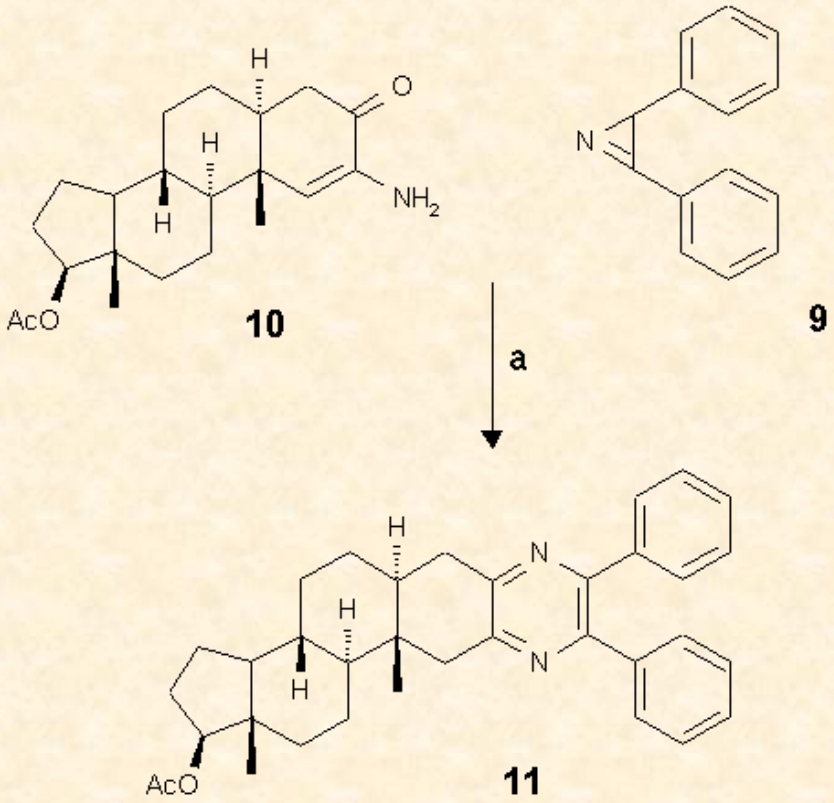
Simultaneous with our own efforts *Clayton Heathcock* [4] developed techniques for a non-symmetric synthesis using a -aminoketone equivalents which needed quite forcing conditions, however.

Looking for a highly reactive a-aminoketone equivalent, we decided to choose azirines as selective reaction partners for the enaminoketones and expected the formation of pyrazine **4b** to follow a reaction path as portrayed in scheme 4.



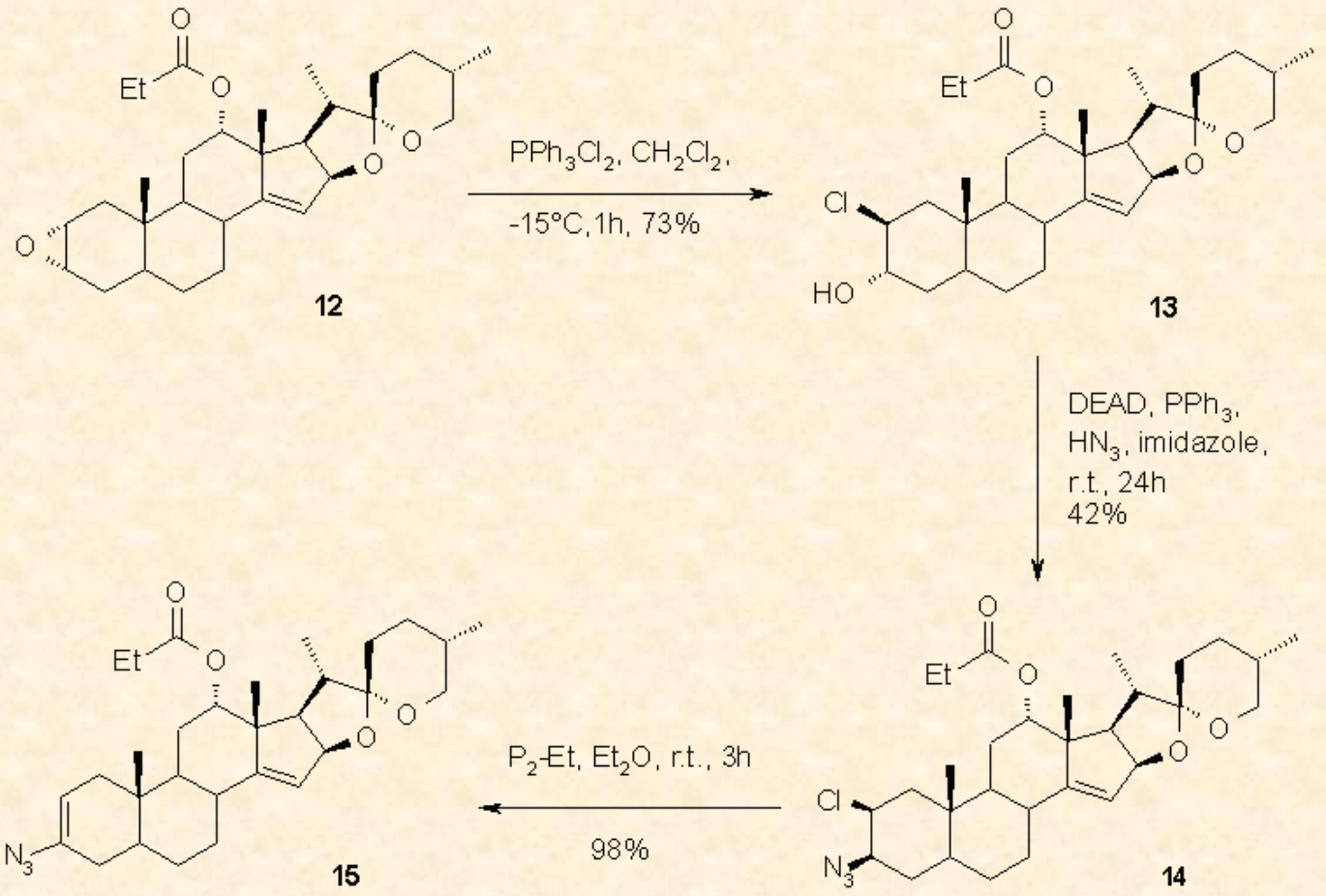
scheme 4: assumed mechanism for pyrazine formation

To check the feasibility of this concept we picked the easily available diphenyl azirin **9** which can be easily prepared from trans-stilbene via iodo-azide addition and subsequent cyclisation according to the *Hassner* protocol [11]. Nucleophilic attack of enaminketone **10** provided the corresponding pyrazine 11 in an acid catalyzed process in 63% yield at 0°C.



scheme 5: non-symmetrical coupling approach to pyrazine 11

Unfortunately, this mild procedure cannot simply be extended to the corresponding steroidal azirines as azirines annellated to six-membered rings cannot be isolated. It has been shown though, that vinyl-azides expel nitrogen on heating or irradiation and can thus operate as precursors for these azirines, we retreated to *Zbiral's* [12] procedure for the formation of 2,3-vinylazides (scheme 6).



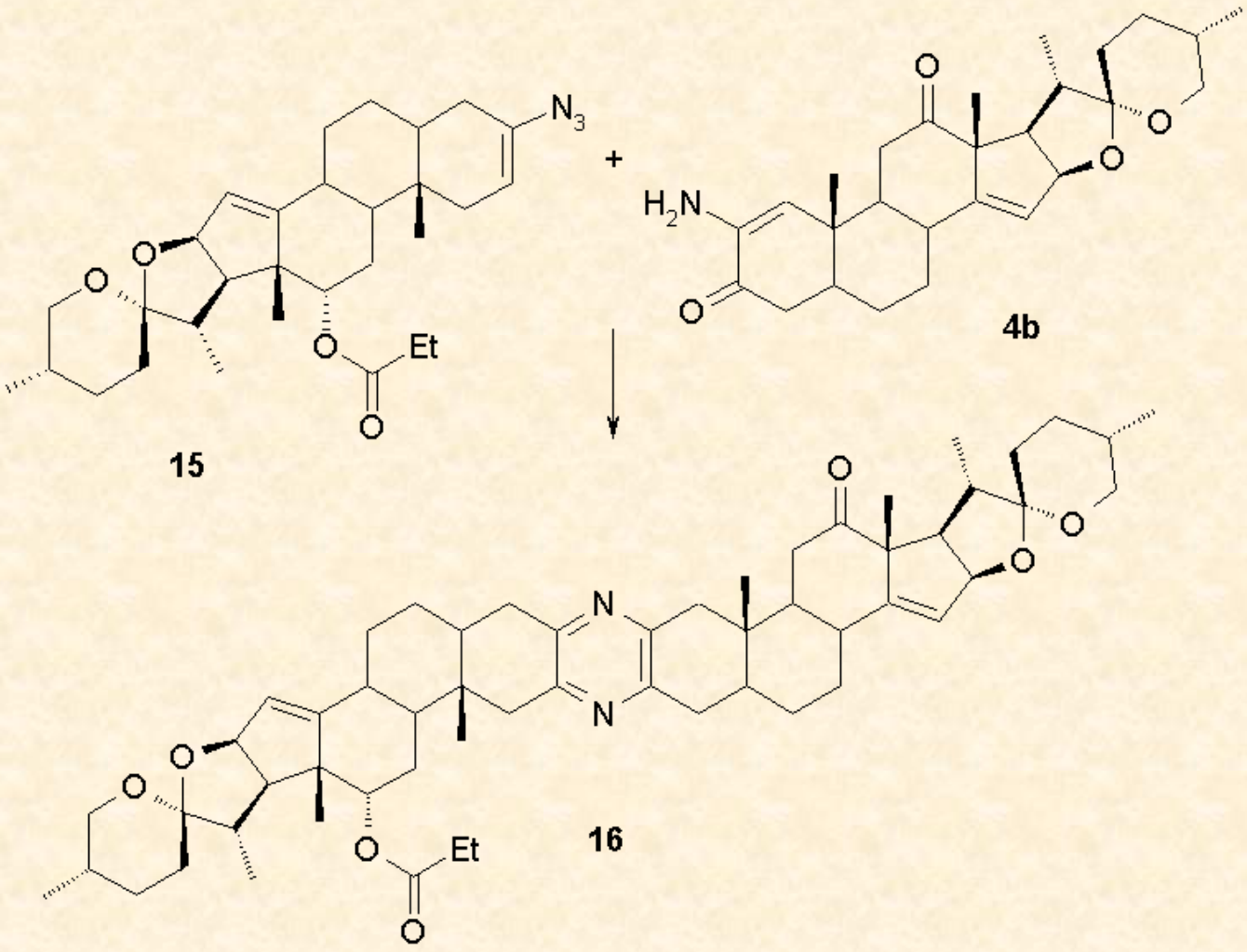
scheme 6: synthesis of vinyl azide 15

The very crucial step in this sequence proved to be the *Mitsunobu* inversion with azide-anions to form chloroazide **14**. In spite of extensive optimization the concurring elimination to form allylic halides, which under the reaction conditions are of course converted into allylic azides, could not be suppressed properly. The final elimination of hydrogen chloride to form the vinylazide produced disappointing results in the beginning too, but when we switched to *Schwesinger's* phosphazene base [13] a 98% yield of the desired compound **15** could be isolated.

Having the vinylazide available various enaminoketones were prepared as described earlier [5,6] and both compounds, which are not forming any homodimers, were refluxed in dioxane in the presence of an acid and 3 Å molecular sieves as a water scavenger. The very efficient binding of any water formed in the process proved to bevery crucial for obtaining acceptable yields in the pyrazine synthesis.

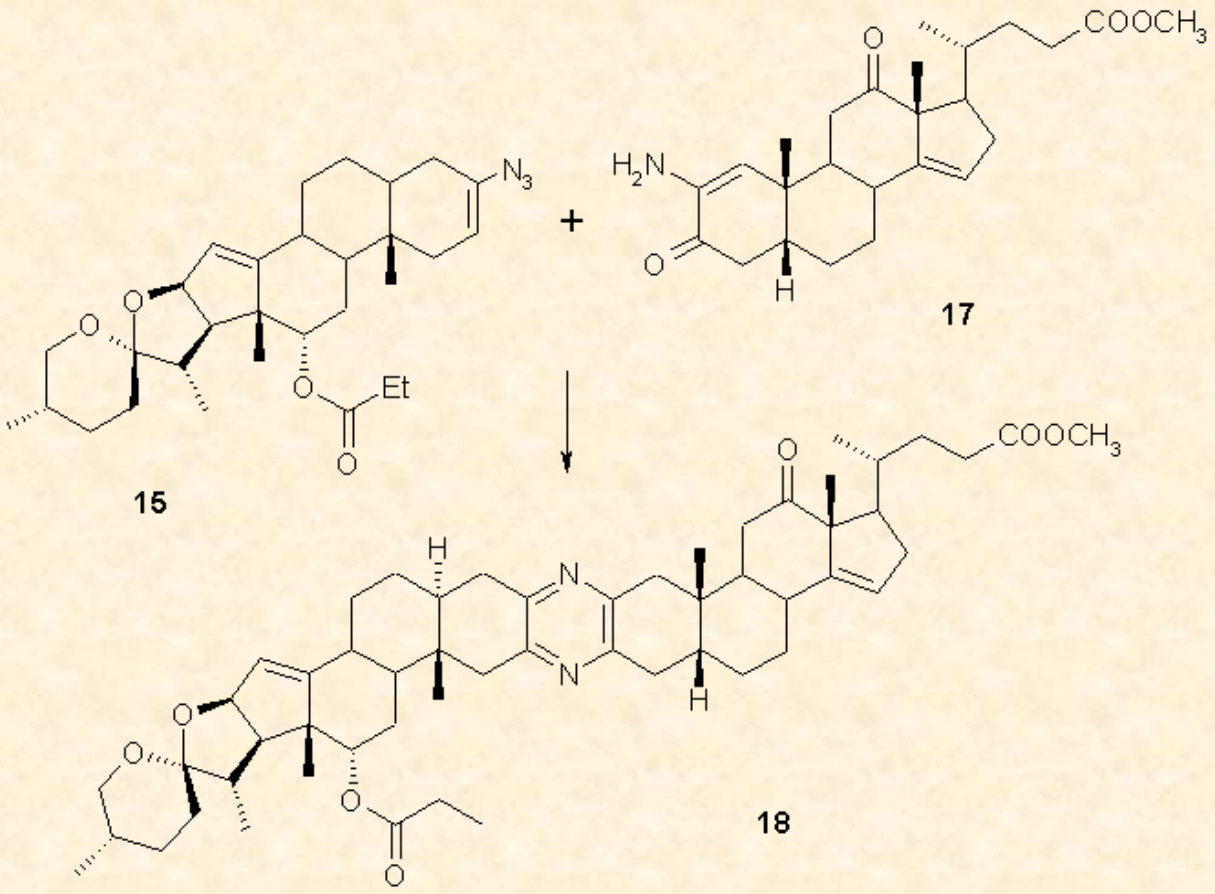
Out of various cephalostatin analogues prepared this way we want to mention three examples, because they prove the process to be quite general and additionally provide compounds that may yield useful informations for the general project.

The condensation of **15** with enaminoketone **4b** gave rise to the non-symmetric pyrazine **16**. This substance can easily be correlated to the a -hydroxyketone **7** which had been obtained in the desymmetrization experiments with diketone **5b**. This way the regioselective reduction predicted in scheme 4 was confirmed and both routes leading to non-symmetric compounds were linked together (scheme 7).



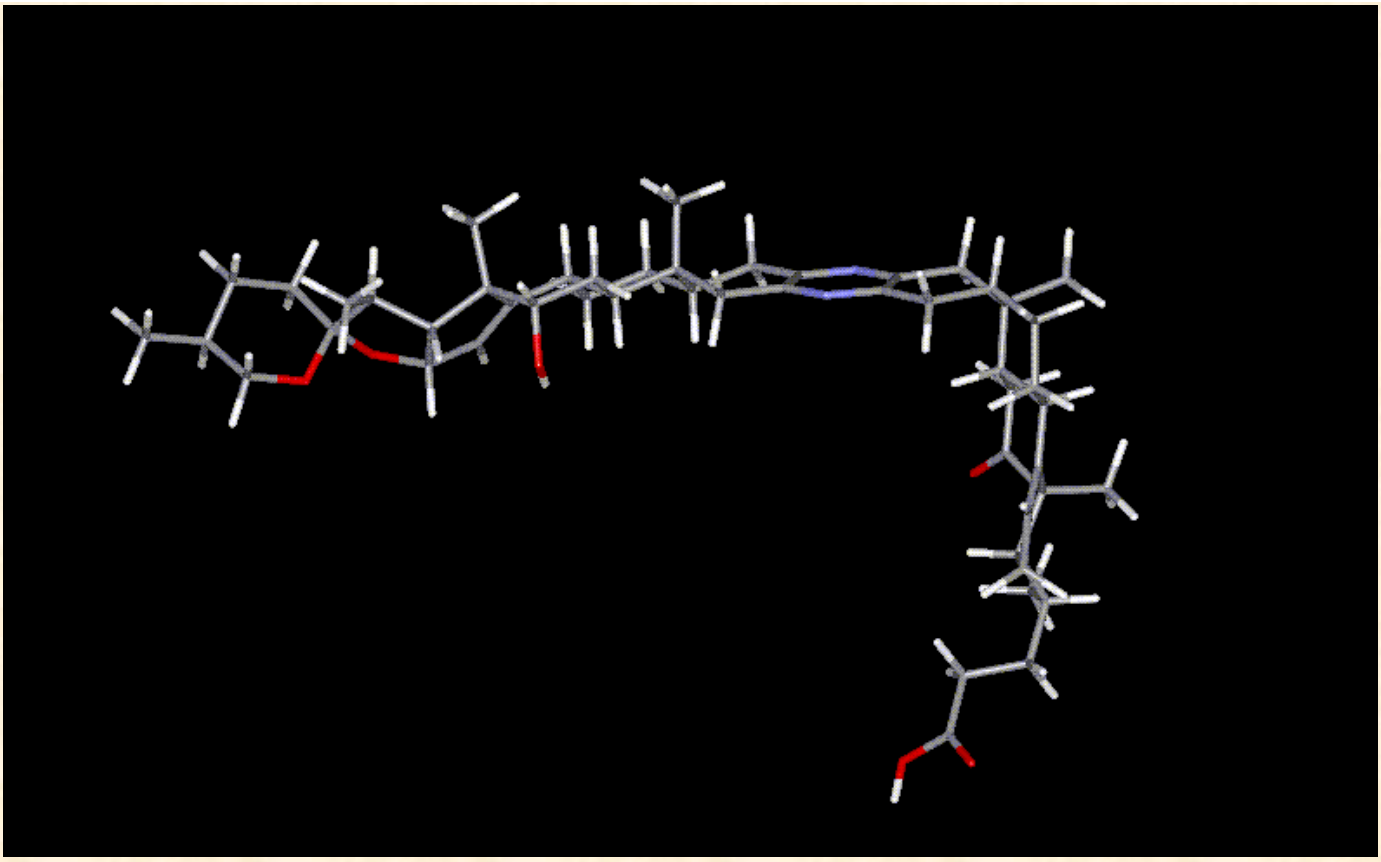
scheme 7: biologically active analogue pyrazine 16

In the second example the vinylazide was combined with the bile-acid derived enaminoketone **1**7 with a cis A-B-ring junction (scheme 8). This leads of course to a complete change of the conformation in this particular bis-steroidal pyrazine. Owing to the cis-configuration in the direct neighborhood of the central aromatic ring, the molecular profile is changed dramatically in comparison to the hydroxyketones **6** and 7 which had been shown to have tumor inhibiting properties.



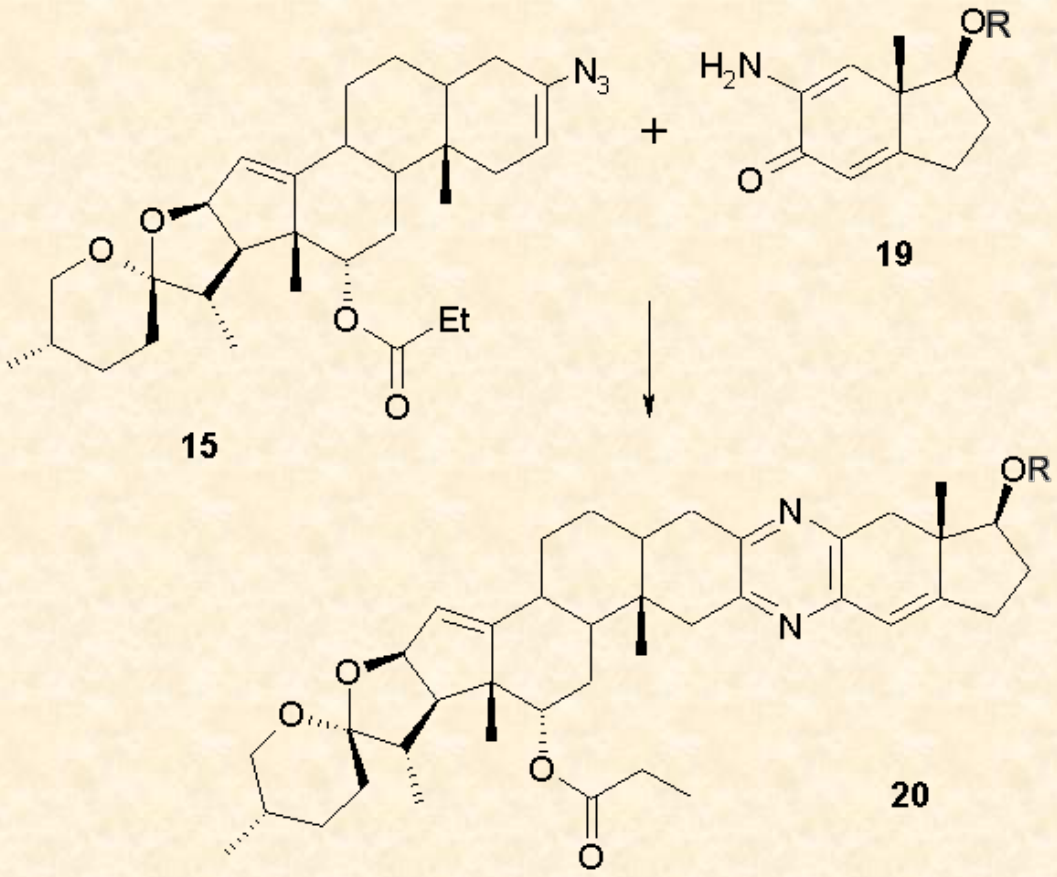
scheme 8: analogue 18 with incorporated cholic acid

The differences are clearly visible from picture 2 and we were therefore not surprised to observe no significant biological activity with these compounds. To prove beyond any doubt that this loss of activity is caused by the bent structure of this material, we are currently preparing the corresponding A,B-trans derivatives which are otherwise absolutely identical as far as constitutional details are concerned.



picture 2: conformational analysis of analogue 18

Having changed the configuration of the alicyclic moiety we finally decided to also change the size of this substructure and therefore prepared enaminoketone **19** from the well known *Hajos-Wiechert* ketone (scheme 9). In the pyrazine obtained in this case at least in the ◆ Eastern◆ part of the cephalostatin analogue the steroid system is reduced to the C-D-hydrindane substructure, but as the testing procedure for compounds of this type is not yet completed, a final judgement cannot be given at the moment. In conclusion one has to state that although both routes the desymmetrysation as well the directed pyrazine formation do provide non-symmetric polycyclic cephalostatine analogues, there still remains the biogenetic problem for the natural occuring compounds.



scheme 9: 'short' analogue pyrazine 20

Unfortunately, both existing methods for a directed preparation of pyrazines are probably not biosynthetic procedures. Which means that either nature has a mild directed synthesis available or she has very efficient means for a highly efficient desymmetrisation process. To test compounds that under marine conditions could well be biosynthetic intermediates and additionally would not form homodimers we investigated the reaction of enaminoketone **4b** with the corresponding a -bromoketone which is actually an intermediate en route to **4b**. This combination can of course form only azapyrrylium salts and so one at a later stage, after the starting materials have been consumed, has to add ammonia to the reaction mixture. Although this transformation was conducted under quite a set of conditions we never observed any pyrazine formation.

Financial support of these investigations by the *Deutsche Forschungsgemeinschaft* in connection with the *Graduiertenkolleg GRK 223* is gratefully acknowledged we also thank *Prof.Dr.U.Eder* and *Dr.H.Laurent* (*Schering AG, Berlin*) for a generous supply of compounds and reagents. M.N. thanks the *DAAD* for a fellowship.

Selected experimental Data:

diphenylpyrazinoandrostane (11): A solution of 150 mg (0.43 mmol) of azirine (**10**), enaminoketone (**9**) and 50 µl of trifluoroacetic acid in degassed THF was stirred at 0 °C for 3 h. After addition of 5 ml of sat. NH_4Cl solution in water the mixture was extracted twice with dichloromethane and dried over $MgSO_4$. The solution was reduced in vacuo a purified by flash chromatography to obtain 140 mg (63%) of diphenylpyrazinoandrostane (**11**). $C_{35}H_{42}N_2O_2$; m.p. 254 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 0.8-1.9 (m, 23 H), 2.05 (s, 3 H), 2.10-2.24 (m, 1 H), 2.48-3.18 (m, 4 H), 4.63 (dd, *J* = 8 Hz, J = 9 Hz, 1 H), 7.22 - 7.31 (m, 6 H), 7.37-7.43 (m, 4 H); ^{13}C-NMR ($CDCl_3$, APT, 50 MHz): δ = 12.07 (-), 12.11 (-), 20.78 (+), 21.18 (-), 23.55 (+), 27.50 (+), 28.30 (+), 31.15 (+), 35.17 (-), 35.62 (+), 35.86 (+), 36.87 (+), 41.89 (-), 42.56 (+), 46.06 (+), 50.63 (-), 53.64 (-),82.76 (-), 128.19 (-), 129.65 (-), 138.98 (+), 139.02 (+), 149.65 (+), 149.82 (+), 149.86 (+), 171.23 (+); IR (KBr, ν_{max}/cm^{-1}): 3060, 2923, 2928, 1734, 1448 w, 1393, 1247, 1029, 699; UV (MeOH): 320, 300 (sh), 248 nm; MS (EI, 150°C): m/z (%) = 522 (39, M^+), 521 (100), 260 (23); HRMS: calcd. 522.3246, found 522.3108; EA calcd. C 80.42, H 8.10, N 5.36; found C 80.24, H 7.78, N 5.82

2-ene-3-azido-12α-propionate (15): 358 mg (0.66 mmol) of chloroazide (**14**) were dissolved in 3 ml of diethylether. 262 µl (0.79 mmol) of phophazene base P_2-Et were added slowly at room temperature by syringe. After 5 h the solvent was evaporated in vacuo and the residue was purified on silica gel to give 307 mg (92%) of the vinylazide (**15**) as a white foam. $C_{30}H_{43}N_3O_4$; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.80 (d, *J* = 6.2 Hz, 3 H, 27-H), 0.80 (s, 3 H, 19-H), 0.98 (d, *J* = 6.8 Hz, 3 H, 21-H), 1.11 (tr, *J* = 7.6 Hz, 3 H, prop-methyl), 1.14 (s, 3 H, 18-H), 0.78-2.1 (m, 33 H), 2.13 (m, 1 H, 8-H), 2.26-2.36 (m, 3 H, 17-H, α-C=O), 3.39-3.53 (m, 2 H, 26-H), 4.83 (dd, *J* = 8.3, 1.8 Hz, 1 H, 16-H), 4.89 (tr, *J* = 2.8 Hz, 1 H, 12-H), 5.17 (m, 1 H, 2-H), 5.46 (m, 1 H, 15-H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 9.29 (q), 11.47 (q), 14.09 (q), 17.15 (q), 18.62 (q), 26.62 (t), 27.96 (t), 28.20 (t), 28.71 (t), 29.12 (t), 30.29 (t), 30.39 (d), 31.22 (t), 34.32 (d), 34.69 (s), 38.84 (t), 41.46 (d), 44.52 (d), 49.68 (d), 50.03 (s), 53.69 (d), 67.16 (t, 26-C), 78.10 (d, 12-C), 85.16 (d, 16-C), 106.66 (s, 22-C), 110.25 (d, 2-C), 120.89 (d, 15-C), 133.75 (s, 3-C), 153.31 (s, 14-C), 173.97 (s, prop-C=O); IR ($CHCl_3$): ν = 3012 (m), 2956 (s), 2928 (s), 2876 (m), 2100 (s), 1724 (s), 1460 (w), 1224 (s); MS (EI, 160 °C): 481 (13, M^+-N_2), 413 (13), 410 (11), 408 (12), 407 (17, M^+-$C_5H_{10}O_2$), 335 (12), 294 (15), 293 (28); HRMS: calcd. 509.3254, found 509.3229; EA calcd. C 70.70, H 8.50; found C 70.47, H 8.43

(((25'R)-12'a-propyloxy-5'a-spirost-14-eno[2,3-b])(24-methyl-5b,14a-chol-14-en-12-on-24-ato[2,3e]))pyrazine (18): 40 mg of 2-enaminoketone (**17**) (0.10 mmol) and 51 mg of 3-vinylazide (**15**) (0.10 mmol) were dissolved in 1 ml of abs. dioxane and appr. 1 mg of PPTS was added. The solution was degassed with argon and 30 mg of 4Å molecular sieves (activated powder) were added. The suspension was refluxed for 2,5 h under argon, allowed to cool to room temperature and filtered through a silica plug with diethylether as eluent. The solvent was removed in vacuo and the crude was flash chromatographed on 4 g of silica gel with a solvent mixture of ethyl acetate / hexanes to obtain 26 mg (30%) of dimer (**18**). $C_{55}H_{76}N_2O_7$; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0,78 - 3 (m, 65 H), 3,45 (m, 2 H, 26'-H) 3,62 (s, 3 H, 24 H), 4,81 (d, 1 H, H-16'), 4,90 (s, 1 H, 12'-H), 5,28 (m, 1 H, 15-H), 5,60 (s, 1 H, 15'-H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 9,3 (q); 11,7 (q); 14,1 (q); 17,2 (q); 17,6 (q); 18,7 (q); 19,1 (q); 21,9 (q); 28,1 (t); 28,7 (t); 29,7 (t); 30,4 (d); 30,5 (t); 31,2 (t); 31,3 (t); 31,7 (t); 31,9 (t); 33,5 (d); 33,6 (t); 33,8 (t); 34,2 (d); 34,4 (d); 34,6 (t); 34,9 (t); 35,5 (s); 35,8 (s); 38,3 (t); 38,7 (s); 38,9 (d); 41,4 (d); 41,7 (d); 42,4 (t); 44,5 (d); 45,6 (t); 46,9 (d); 47,0 (d); 49,7 (d); 50,0 (s); 51,5 (q); 52,9 (d); 53,6 (d); 62,7 (s); 67,3 (t); 78,0 (d); 85,2 (d); 106,8 (s); 120,5 (d); 121,1 (d); 147,3 (s); 148,5 (s); 148,6 (s); 149,0 (s); 151,6 (s); 153,4 (s); 173,9 (s); 176,8 (s), 213.4 (s); IR: 1724, 1672, 1460, 1400; FAB: 878 (MH^+); HR-FAB (MH^+) calcd: 877,5731, found 877,5838; UV: 305 (sh), 288 nm

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