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Synthesis and biological activity of analogues of ptilomycalin A

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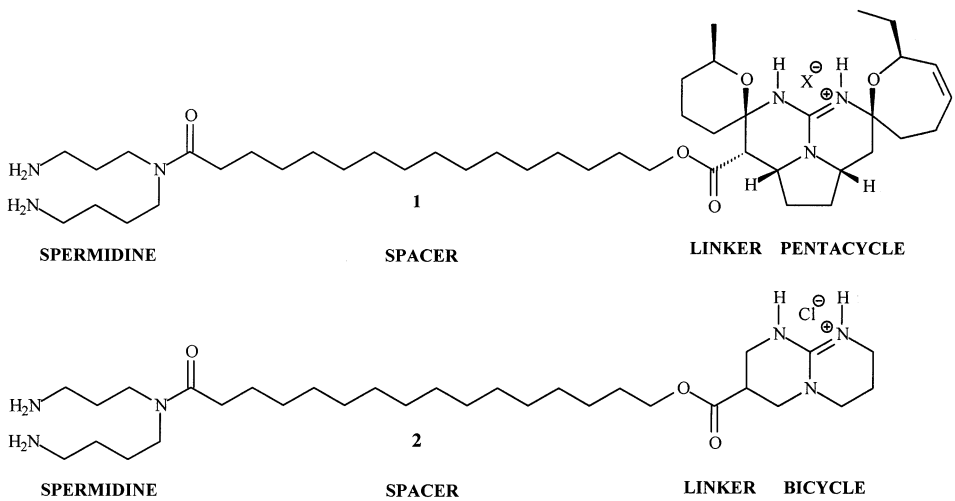
Abstract—Benzo-fused model compounds **21a** and **21b**, resembling in structure the marine metabolite ptilomycalin A, were prepared and were shown to display significant activity against a series of cancer cell lines and to also possess a significant activity against the DNA polymerase activity of the reverse transcriptase of human immunodeficiency virus type 1 (HIV-1 RT). © 2001 Elsevier Science Ltd. All rights reserved.

Ptilomycalin A **1** and related natural products have been the subject of considerable synthetic interest owing to their high levels of diverse biological activity. This includes cytotoxicity towards several cancer cell lines as well as antifungal and antiviral activities.[1] Despite this range of activity, only one report of the synthesis of a structural analogue of ptilomycalin A has been reported, by Hart and Grillot, which detailed the preparation of the racemic bicyclic guanidine **2**[2] (Scheme 1).

As can be seen, ptilomycalin A can be considered as four separate components, a guanidine-containing polycyclic core, an ester linkage to an ω-hydroxyacid spacer unit and a terminal spermidine residue. In common



Scheme 1.

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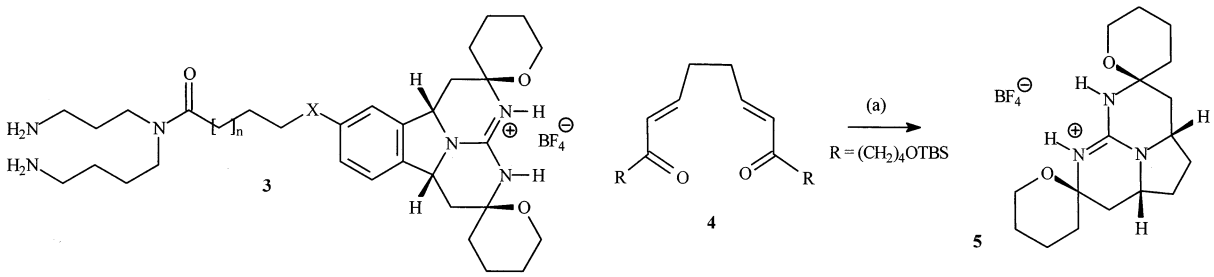


Scheme 2. (a) (i) Guanidine, DMF, 3 h; (ii) MeOH, HCl, 0°C to rt, 24 h; (iii) $NaBF_4$ (satd, aq.), (25–35%). n = Variable, X = CH_2, O.

with this, the analogue **2** was designed to mimic the natural material **1** with one exception in that it was based upon a much simpler bicyclic guanidine core.[2] Unfortunately, it was reported that **2** was inherently unstable and over a period of a few weeks completely decomposed via an unidentified process which prevented the biological evaluation of this model compound. The structure of the decomposition products were not fully determined, although NMR spectroscopic analysis revealed that the signal due to the methylene group adjacent to the ester oxygen was no longer present. This observation led the authors to speculate that the role of the spiro *N*,*O*-acetal groups in the natural product **1** is to sterically protect the ester linkage from hydrolysis or ammonolysis.[2] As part of our general synthetic programme in this area of chemistry, we were interested in preparing analogues of **1** to evaluate the structural features that are necessary to impart biological activity. The preliminary results of this work are outlined in this communication.

Our strategy was to follow the basic design of the natural molecule but to replace the potentially labile ester linkage with a more stable function. It was envisaged that model compounds of general structure **3**, possessing a benzo-fuzed pentacyclic guanidine group could be prepared in a similar manner to substances reported in our model studies on the synthesis of ptilomycalin A. These studies detailed the preparation of pentacycles such as **5** from the bis-enone **4** by sequential addition of guanidine, deprotection and spirocyclisation[3] (Scheme 2).

It was envisaged that the linker group in **3** would be based on either an ether linkage (X = O) or an alkylated benzene (X = CH_2). The preparation of **3** in a convergent manner would offer the possibility of varying the chain length between the guanidine and spermidine moieties, thereby enabling an investigation of the role of this parameter in the biological activity of ptilomycalin A and related metabolites. We firstly needed to determine if it was indeed possible to prepare the required benzo-fuzed guanidine pentacycle and thus reacted phthaldehyde **6** with the previously reported phosphorane **7**[3] leading to the bis-enone **8** in 85% yield. Reaction of this with guanidine under our standard conditions routinely produced the desired pentacyclic guanidine **9** in 30–40% overall yield as a crystalline compound. From NMR analysis of the crude reaction mixtures it was also apparent that this material was formed as a single diastereoisomer and X-ray analysis† of **9** confirmed the relative stereochemistry to be identical to that found in ptilomycalin A. Encouragingly, the fluoroborate anion was found to interact with the guanidinium ion in a bidentate ligating mode similar to that observed with carboxylates and phosphates (Fig. 1: The data for the N-H···F hydrogen bonds, H···F, N···F distances and N-H···F angles are 2.19/2.06 Å, 2.99/2.92 Å and 155.72/174.57°, respectively).[4] The relative stereochemistry of the molecule is a key factor in the synthesis of analogues of **1** as it is known that disruption of the all *cis* arrangement found in ptilomycalin A has a considerable effect on biological activity[1] (Scheme 3).

After several unsuccessful attempts at derivatising the aromatic ring of **9** by electrophilic substitution, we decided to adopt a more direct approach by preparing 4-substituted phthaldehydes which could be modified to the required model compounds. One reported[5] preparation of 4-substituted phthaldehydes is via ozonolysis of 2-substituted naphthalenes and hence we considered naphthalene **13** to be a suitable precursor. Isomerisation of the alkyne in commercially available 7-hexadecyn-1-ol **10** to the terminus by means of the 'Zipper' reaction,[6] followed by silyl protection of the alcohol group gave **11** in good overall yield. This was then converted into the corresponding vinyl boronic acid using catecholborane and coupled to 2-bromo-6-methoxynaphthalene under Suzuki conditions to give alkene **12** in 78% yield. Hydrogenation of the alkene group under standard conditions gave the required substrate **13** in 91% yield. The key reaction of **13** with

† *Crystal data* for $C_{21}H_{28}N_3O_2{\cdot}BF_4{\cdot}CHCl_3$: M_r = 500.45, monoclinic, a = 18.303(3), b = 16.421(2), c = 17.961(4) Å, β = 119.30(6)°, U = 4707.6(11) Å^3, space group $C2/c$, Z = 8, D_c = 1.412 g cm^{-3}, $F(000)$ = 2084, (Mo Kα) = 0.275 mm^{-1}. Data were collected at 150(2) K, for a crystal of dimensions 0.23×0.22×0.17 mm, on a FAST TV Area detector diffractometer following previously described procedures.[10a] A total of 9061 data were recorded (index ranges = −19h19, −15k17, −19l19) and merged to give 3049 unique reflections (R_{int} = 0.0723). The structure was solved via direct methods (SHELX-S)[10b] and then refined by full matrix least-squares on all F^2_o data (SHELX-93).[10c] The final R, R_w indices [I>2(I)] were 0.0797, 0.1935 and 0.1123, 0.2055 for all data, respectively, with 367 parameters. Full details of the data collection, structure refinement, atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (Deposition Number = 148301).

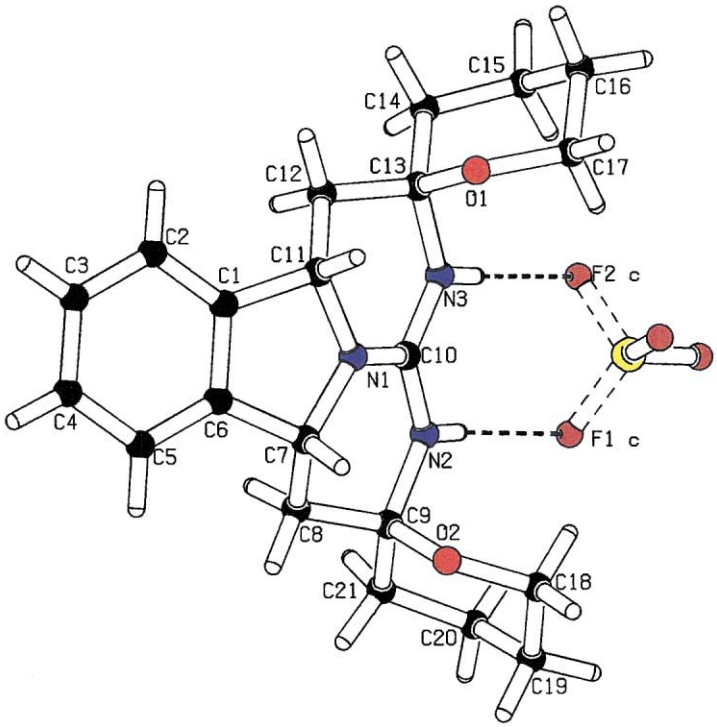
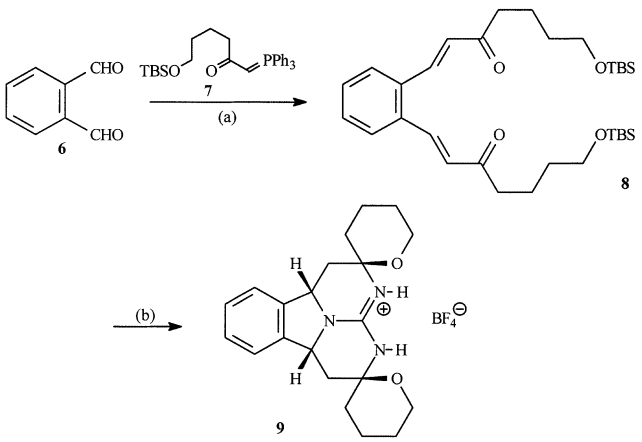


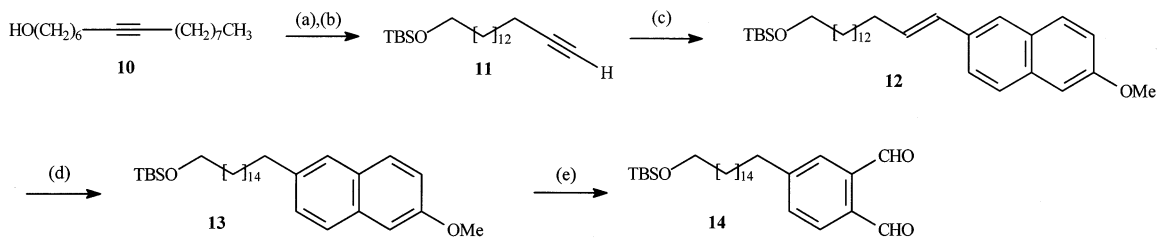
Figure 1.



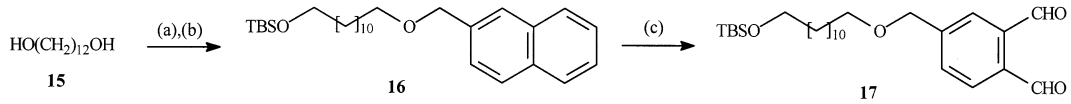
Scheme 3. (a) CH_2Cl_2, reflux, 6 h, (85%); (b) (i) DMF, guanidine, 0–20°C, 5 h; (ii) MeOH, HCl, 0°C, 1 h, then 20°C, 15 h; (iii) $NaBF_4$ (satd, aq.); (iv) trituration and crystallisation, (30–40%).

ozone under the conditions described by Pappas et al.[5] did indeed lead to selective ozonolysis of the methoxy-substituted ring, however, the yields for this process were not as high as those reported for the simpler substrates described in the original work. Typical yields of phthaldehyde **14** were of the order of 20–25% which could be improved by stopping the reaction at ca. 50% completion and recycling the recovered starting material, eventually leading to an overall yield of 40% (Scheme 4).

We also investigated a simpler approach to the preparation of a 4-substituted phthaldehyde and prepared **17** via a convenient three-step route which involved alkylation of diol **15** using 2-bromomethylnaphthalene, followed by silyl protection of the remaining alcohol leading to **16** in high yield. Ozonolysis of **16** led to the dialdehyde **17**, but again the yields for this step were poor (20–30%), however, the ease of preparation of the precursors enabled the synthesis of gram quantities for further study (Scheme 5).



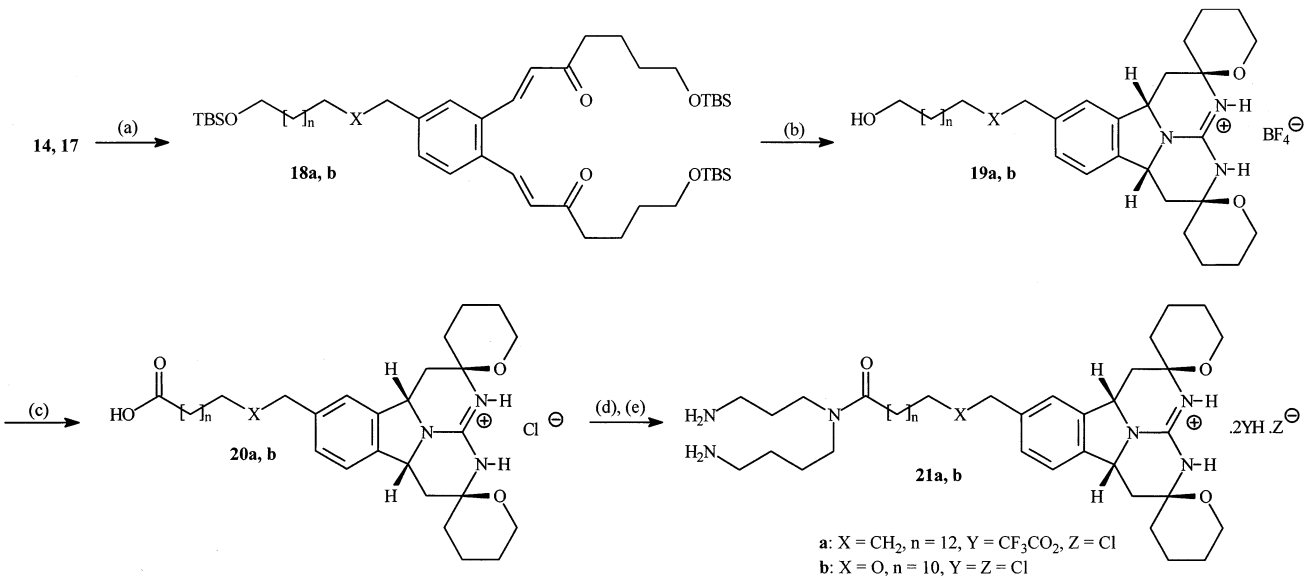
Scheme 4. (a) Li, 1,3-diaminopropane, 70°C, KO*t*-Bu, Et_2O, (72%); (b) Imid, TBSCl, DMF, 2.5 h, (98%); (c) (i) catecholborane, reflux, 3 h, rt, 16 h; (ii) PhH, Pd $(PPh_3)_4$, 2-bromo-6-methoxynaphthalene, 2.5 h, then NaOEt/EtOH (2 M), reflux, 2 h; (iii) NaOH (3 M), rt, 16 h, (78%); (d) EtOAc, Pd/C, H_2, 10 min, (91%); (e) CH_2Cl_2, O_3, −78°C then PPh_3, (40%).



Scheme 5. (a) NaH, THF, 2-bromomethylnaphthalene, (64%); (b) Imid, TBSCl, DMF, (93%); (c) CH_2Cl_2, O_3, −78°C then PPh_3, (20–30%).

With the two aldehydes **14** and **17** in hand we proceeded to the synthesis of the analogues by reacting them with the phosphorane **7** under standard conditions to yield the enones **18a** and **18b** in 70 and 76% yield, respectively. Addition of guanidine was accomplished as before to give **19a** in 25% yield and **19b** in 28% yield, which are typical yields for this reaction. Analysis of the proton and carbon NMR spectra for these substances indicated that they gave near identical signals to compound **9** and had also been formed as single diastereomers. Oxidation of the terminal hydroxyl groups was accomplished using PDC/DMF[7] in 54 and 85% yields, respectively, and coupling of the carboxylic acids with bis-Boc protected spermidine[8] using EDCl proceeded in 88 and 87% yield for the two substrates. Finally, acidic deprotection of the Boc groups gave the required analogues **21a** and **21b** in quantitative yields (Scheme 6).

The compounds **21a** and **21b** were tested against four cancer cell lines and also for their capacity to inhibit the enzyme reverse transcriptase of human immunodeficiency virus type 1 (HIV-1 RT)[9] (Table 1). Of the two analogues, compound **21a** showed by far the best activity against all the cell lines studied, demonstrating an IC_{50} activity at less than 1 μg ml^{-1} in each case, and also displayed 64% inhibition of HIV-1 RT. Compound **21b** also showed activity against the same cell lines but to a lesser extent than **21a**, together with 55% inhibition of HIV-1 RT. Comparison of these results with those obtained from ptilomycalin A indicated that the activity of **21a** was comparable in magnitude to the natural material for all the cell lines studied and that both **21a** and **21b** gave near identical levels of HIV-1 RT inhibition to **1**. The levels of activity for the simple benzo analogue **9** were also investigated and this proved to be



Scheme 6. (a) 3 equiv. **7**, CH_2Cl_2, 24–48 h, (70, 76%); (b) (i) guanidine, DMF, 7 h, 0°C to rt; (ii) MeOH, HCl, 0°C to rt, 24 h; (iii) $NaBF_4$ (satd, aq.), (25, 28%); (c) PDC, DMF, (54, 85%); (d) bis-(*N*-1, *N*-8)-Boc-spermidine, EDCl, HOBT; (88, 87%); (e) for **21a**: $CHCl_3$, CF_3CO_2H; for **21b**: HCl (3N, aq.); (quant.).

Table 1.

Compound	K562[a]	A2780[a]	H-460[a]	P388[a]	HIV-1 RT[b] (%)
Ptilomycalin A **1**	0.35	0.27	0.35	0.11[c]	60
21a	0.52	0.92	0.52	0.69	64
21b	7.28	11.02	5.87	4.25	55
9	24.93	22.06	21.36	9.11	0

[a] Cytotoxic activity (IC_{50}/μg ml^{-1}); K562: human chronic myelogenous leukaemia; A2780: human ovarian carcinoma; H-460: human large cell carcinoma; lung. High DT-diaphorase; P388: mouse, lymphoid neoplasm.

[b] Percentage of inhibition of DNA polymerase activity of HIV-1 reverse transcriptase in the presence of final concentrations of 10 μM inhibitor.

[c] Data obtained from Refs. 1a and 1b.

the least active compound of the three, which indicates that the presence of a spacer chain and spermidine residue are essential for the compounds to be able to demonstrate significant biological activity. It is also apparent that inclusion of the ester linkage is not necessary for activity and that the obvious changes in the overall geometric arrangement of the guanidine and spermidine residues has not affected the levels of activity to a great extent.

In conclusion, we have successfully prepared two novel benzo-fused pentacyclic analogues of ptilomycalin and have shown that both compounds display significant activity against a series of cell lines and also HIV-1 RT inhibitory activity. Drawbacks in the synthesis of these compounds were the poor yields observed for the ozonolysis of the naphthalene intermediates leading to the 4-substituted phthaldehydes. In spite of this we are currently investigating alternative methods for the synthesis of these analogues and intend to use this work as a platform to prepare a series of compounds in which variations in length of the alkyl spacer are introduced to ascertain any effects this might have on biological activity.

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