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Synthesis and characterisation of a new podand based on a calixarene and a β-lactam

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Abstract—Two penicillin arms have been grafted at the lower rim of the *p-tert*-butylcalix[4]arene, giving a new kind of podand which was fully characterised.

The calixarenes have often been employed in recent years as carriers and spatial organisers of various kinds of active substituent, displaying properties dealing with recognition of organic substrates or metallic cations. Efforts have also been brought to the use of their specific conformations in order to prepare, notably in the case of the tensed calix[4]arene, highly ordered and organised molecular devices.[1] The expectation of sophisticated properties related to the high degree of organisation they are able to provide could nevertheless hide their ability to act as simple carriers, particularly in the medicinal field. We found that very few reports, essentially patents, have been devoted to the study of calixarenes as intrinsically active therapeutic agents, such as *para*-sulfonato-, phophonato- or other hydrophilic analogues, against bacteria, fungi, cancerous cells and viruses,[2] thrombosis,[3] infection by enveloped viruses[4] or fibrosic diseases.[5] Some reports are attached to the study of the 'old' Macrocyclon[6] and analogues in the treatment of tuberculosis and other mycobacterioses,[7] or to the building of antimicrobial calixarene-based vancomymin mimics.[8]

In order to develop calixarene-based podands shaped as potent drug dispensers, we initiated a study related to the grafting of a simple and easily characterisable drug subunit displaying unambiguous analytical characteristics. Our first molecular target was an antibiotic penicillin grafted in alternate positions of the cone conformer of the *p-tert*-butylcalix[4]arene.

The synthetic strategy involved the formation of a hemi-synthetic penicillin via the formation of an amide bound between the calixarene platform and the penicillin moiety. The latter was protected at the carboxy group by a pivaloyloxymethyl ester function, thus giving a lipophilic prodrug which should liberate the biologically active free acid after hydrolysis by esterases, as initially developed with the commercial pivmecillinam and pivampicillin.[9] The choice of the coupling reaction involved soft conditions in order to avoid probable degradation of the β-lactam ring. Thus, a previously described controlled peptide-bound formation process was chosen.[10] The diacid **1** was reacted with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide in dry CH_2Cl_2 to give the corresponding bis-activated ester **2** with a yield of 85%.[11] The penicillin derivative **3** was prepared as the tosylate salt in the conditions described by Daehne et al.,[9] Matlin et al.[12] or Ogura et al.,[13] by reaction of pivaloyloxymethyl chloride and 6-aminopenicillanic acid.

The last step consisted of the reaction of **2** and **3** in mild conditions, in CH_2Cl_2 at rt under argon. The bis-penicillin podand **4** was obtained with a good degree of purity (>95%) after chromatography with a yield of ca. 70%. An analytical sample was obtained using precise thin-layer preparative chromatography (Scheme 1).

Positive mode electrospray mass spectrometry confirmed that the desired compound was obtained with a base peak at 1411.59 a.m.u., attributed to $[M+Na]^+$, while the negative mode gave a base peak at 1387.86 a.m.u., attributed to $[M-H]^-$, accompanied by various fragmentation products.

IR analysis showed the presence of three intense bands: one at 1693 cm^{-1}, which was attributed to the amide

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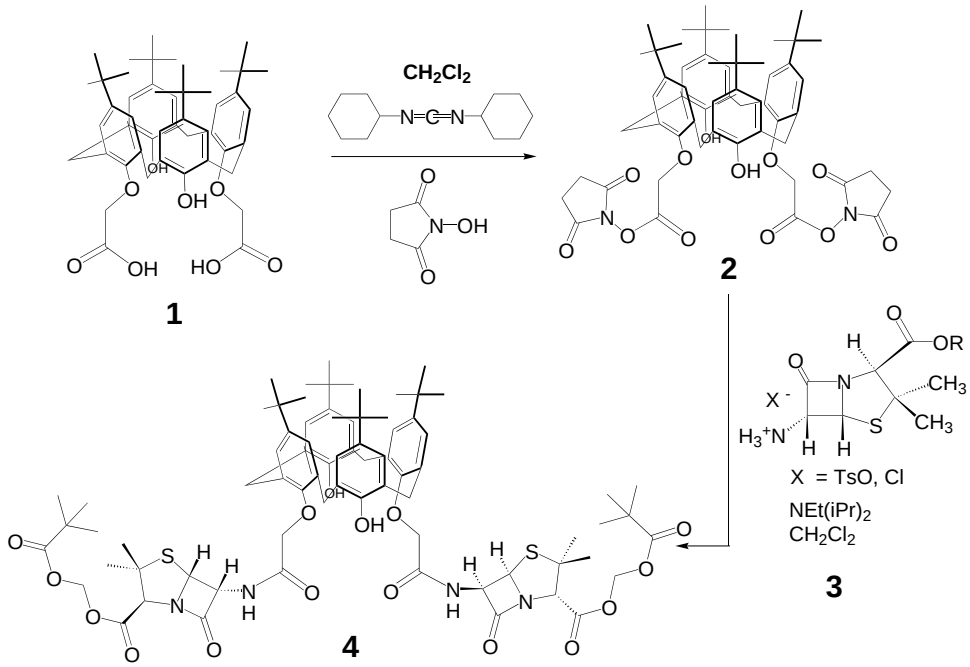

Scheme 1.

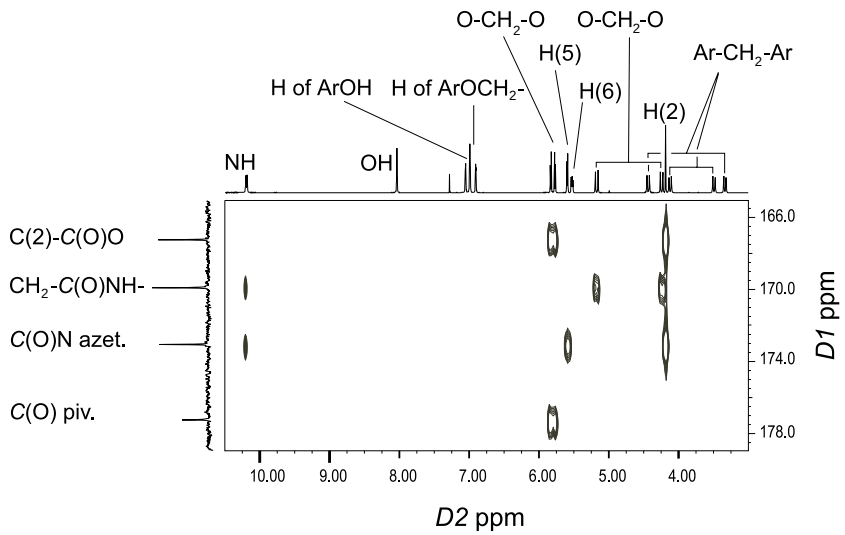

Figure 1. ^{1}H, ^{13}C HMBC experiment ($CDCl_3$, 400 MHz, rt) in the carbonyl region of **4**.

carbonyl group, and of two close bands at 1759 and 1792 cm^{-1}, which were attributed without more precision to the constrained lactam carbonyl group and to the esters.

^{1}H and ^{13} C NMR analyses showed the presence of the specific resonance signals of both calixarene and penicilline moieties. Nevertheless, the full attribution of these signals necessitated the help of 2D-COSY and HMBC techniques.

According to de Mendoza et al.,[14] **4** is in a distorted cone conformation, as assessed by the Ar-CH_2-Ar resonance signals at 32.44 and 33.38 ppm. Another distortion appears in the aromatic part of the spectrum, which shows the presence of four resonance signals for the calixarenic *ortho*-carbons. This phenomenon can be related to a probable helical arrangement of the two chiral pendant penicillin arms along the main axis of the calixarene. The ^{1}H NMR spectrum confirmed this hypothesis through the presence of an expanded AB system at 4.24–5.17 ppm ($J_{AB}=14.7$ Hz). Unambiguously attributed to the acetyl -CH_2- protons, the latter was justified, as previously proposed,[9] by hydrogen bondings between the free OH groups and the close amide carbonyl subunits. Due to this helicity, the two methyl groups at the 3-position of the penicillin moieties were strongly discriminated, passing from 1.42 and 1.49 ppm in **3**, to 0.90 and 1.38 ppm in **4**. The azetidin-2-one protons H(6) and H(5) and the external amide

appear as a perfect suit of resonance signals: a dedoubled doublet at 5.52 ppm ($J_1=6.5$ Hz, $J_2=3.8$ Hz), a doublet at 5.59 ppm ($J=3.8$ Hz) and a doublet at 10.19 ppm ($J=6.7$ Hz), respectively. The methylene protons of the pivaloyl groups appear as a well-defined AB system ($J_{AB}=5.3$ Hz) at 5.77, 5.83 ppm, their non-equivalence probably resulting from their chiral and hindered environment. Finally, the four carbonyl resonance signals were assigned by $^1H,^{13}C$ HMBC experiment (Fig. 1).

The penicillinic podand **4** described here is representative of a new kind of potentially therapeutically active calixarene derivatives; for this reason, its antibiotic activity as well as the synthesis of its mono-, tri- and tetra-substituted analogues are under current investigation.

Acknowledgements

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11. **Compound 2**: A suspension of diacid **1** (0.206 g, 2.7 10^{-4} mol), *N*-hydroxysuccinimide (0.068 g, 5.92 10^{-4} mol) and dicyclohexylcarbodiimide (0.122 g, 5.92 10^{-4} mol) in ethyl acetate (40 ml) was stirred at 25°C under argon for 48 h. The precipitated urea was filtered off and the filtrate was evaporated to dryness to give **2** as a white powder (0.22 g; 85%). ^1H NMR ($CDCl_3$): 0.93 (s, 18H, $2Me_3C$); 1.31 (s, 18H, $2Me_3C$); 2.88 (s, 8H, $2CH_2CH_2$); 3.39, 4.39 (AB, $J_{AB}=13.4$, 8H, Ar-CH_2-Ar); 5.21 (s, 4H, $2OCH_2$); 6.59 (s, 2H, 2 O*H*); 6.74 (s, 4H, Ar*H*); 7.08 (s, 4H, Ar*H*). Identical to Ref. 10. **Compound 3**: *N*,*N*-diisopropylethylamine (0.61 ml, 3.5×10^{-3} mol) was added to a suspension of 6-APA (0.55 g, 2.53×10^{-3} mol) in dry DMF (2.5 ml; dried and distilled over CaH_2 at rt) and the mixture was stirred at rt under argon for 30 min. Chloromethyl pivalate (0.74 ml, 5×10^{-3} mol) was then added and stirring was continued at 26–28°C for 4 h. Ethyl acetate (20 ml) was added and the resulting precipitate was filtered off. The filtrate was washed with H_2O (3×5 ml), dried over $MgSO_4$ then concentrated to a half. A pH-controlled addition of a solution of *p*-TsOH in ethyl acetate (0.475 g, 2.5×10^{-3} mol, 4.5 ml) resulted in the slow precipitation of POMAPATs, which was collected as white crystals (0.5 g; 40%). ^1H NMR: 1.05 (s, 9H, Me_3C); 1.42 (s, 3H, CH_3); 1.49 (s, 3H, CH_3); 2.37 (s, 3H, CH_3 of Ts); 4.47 (s, 1H, H(2)); 4.98 (d, $J=4.2$ Hz, 1H, H(5) or H(6)); 5.41 (d, $J=4.2$ Hz, 1H, H(6) or H(5)); 5.75, 5.84 (AB, $J_{AB}=5.5$, 2H, OCH_2O); 7.17 (d, $J=8$, 2H, Ar*H*); 7.79 (d, $J=8.2$, 2H, Ar*H*). Identical to literature. **Compound 4**: A solution of **3** (0.115 g, 2.29×10^{-4} mol) and diisopropylethylamine (0.11 ml, 6.32×10^{-4} mol) in CH_2Cl_2 (7 ml) was added to a solution of the activated ester **2** (0.1 g, 1.07×10^{-4} mol) in CH_2Cl_2 (7 ml) and the mixture was stirred at 25°C under argon for 16 h. A second portion of **3** (0.5 equiv.; 0.026 g, 0.52×10^{-5} mol) was then added and stirring was continued for 4 h. The solvent was evaporated to dryness without heating and the resulting solid material was chromatographed (SiO_2; CH_2Cl_2:CH_3CN, 90:10) to give an analytical sample of **4** as a white powder (0.056 g; 37%). Mp: 150–152°C. UV ($CHCl_3$): 284 (6000). IR (KBr):1693 (CO amide); 1759, 1792 (strong and sharp, CO lactam, CO esters). ^1H NMR: 0.90 (s, 6H, CH_3 of APA); 1.08 (s, 18H, Me_3C); 1.22 (s, 18H, Me_3C of Piv.); 1.25 (s, 18H, Me_3C); 1.38 (s, 6H, CH_3 of APA); 3.33, 4.43 (AB, $J_{AB}=13$ Hz, 4H, Ar-CH_2-Ar); 3.49, 4.11 (AB, $J_{AB}=14$ Hz, 4H, Ar-CH_2-Ar); 4.18 (s, 2H, H(2) of APA); 4.24, 5.17 (AB, $J_{AB}=14.7$ Hz, 4H, OCH_2); 5.52 dd, $J_1=6.5$ Hz, $J_2=3.8$ Hz, 2H, H(6) of APA); 5.59 (d, $J=3.8$ Hz, 2H, H(5) of APA); 5.77, 5.83 (AB, $J_{AB}=5.3$ Hz, 4H, OCH_2O); 6.90, 6.99 (AB, $J_{AB}=2.9$ Hz, 4H, H(3) and H(5) of $ArOCH_2$-); 6.99, 7.05 (AB, $J_{AB}=2.9$ Hz, 4H, H(3) and H(5) of ArOH); 8.03 (s, 2H, O*H*); 10.19 (d, $J=6.7$ Hz, 2H, N*H*). ^{13}C NMR ($CDCl_3$; 400 MHz): 27.30 (Me_3C piv.); 27.70, 29.78 (*Me* of APA); 31.41, 32.00 (Me_3C); 32.44, 33.38 (Ar-CH_2-Ar); 34.21, 34.54 (Me_3C); 39.20 (Me_3C piv.); 60.06 (C(6) of APA); 64.92 (C(3) of APA); 69.05 (C(5) of APA); 70.81 (C(2) of

APA); 75.40 (O-*C*H_2); 80.21 (O-*C*H_2-O); 125.27, 125.77, 126.44, 127.43 (*C*H of Ar); 126.20, 127.80, 132.89, 133.02 ($C_{(o)}$, $C_{(o')}$ of Ar); 143.16, 148.58 ($C_{(p)}$ of Ar); 149.61, 150.06 ($C_{(i)}$ of Ar); 167.18 (C_1-*C*OO); 169.87 (CH_2*C*ONH); 173.05 (*C*O lactam) 177.28 (*C*O piv.). ES-MS (pos. mod.): 1411.59 $[M+Na^+]^+$. ES-MS (neg. mod.): 1387.68 $[M-H^+]^-$. Anal. calcd for $C_{72}H_{100}N_4O_{16}S_2$, 0.25 CH_2Cl_2 (1411.0): C, 64.91; H, 7.18; N, 3.97; found: C, 64.79; H, 7.13; N, 4.03.

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