SHORT PAPER

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A novel hemisynthesis of the 19-demethyl analogue of calcimycin (or A23187)†

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The hemisynthesis of 19-demethylcalcimycin **8** was carried out in five steps from the naturally occurring calcium ionophore using retroaldol/aldol reactions on an open chain dithioketal intermediate. The key retroaldol cleavage was achieved by using the coupled reagents $Ti(OtBu)_4$ / benzaldehyde.

Keywords: hemisynthesis, A23187, retroaldol/aldol reactions, titanium alkoxides, spiroketalisation

Calcimycin or A23187, a member of the large family of carboxylic polyether antibiotics[1] was originally isolated from *Streptomyces chartreusis* (NRRL 3882).[2] Its specific transmembrane calcium carrier properties[3] has attracted considerable attention in biology as a tool for the study of the calcium second messenger in living systems.[4]

The trimeric neutral association formed with divalent cations (2 ligands^{2-} / 1 M^{2+}), which is the key species in the membrane transport process, have been studied in the solid state or in solution. It appeared that the benzoxazole moiety had rotational mobility around the C_9–C_{10} bond on comparing the acid form[2,7] and the neutral complexes[5–7] (Fig. 1), whereas a preferential conformation for the C_{18}–C_{19} bond, which governs the orientation of the keto-pyrrole arm, was retained, with H_{18} and H_{19} remaining antiperiplanar in all cases.

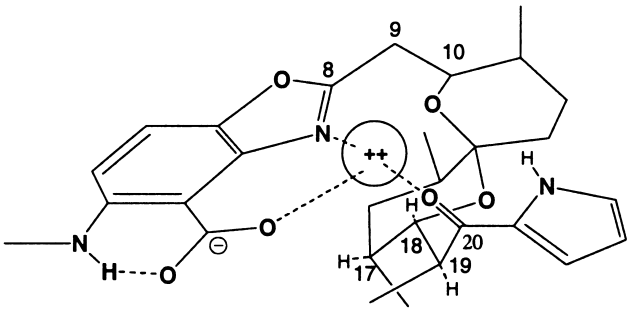

Fig. 1 Schematic representation of an M^{2+} complex with the three coordinating sites. The second ligand is omitted for clarity.

We recently investigated the conformational mobility around the C_{18}–C_{19} bond, using the MacroModel® Program[8], for calcimycin and its analogue where 19(*S*)–CH_3 group is replaced by an H atom. The natural structure showed two significant minima (Fig. 2) corresponding to conformers A and B. They were separated by a high rotational barrier. Conformer A was very close to that observed for this part of the molecule in the quoted structural results.[2,5–7] For the 19-demethyl analog the curve was clearly modified with a less stable A conformer and a significantly lowered rotational barrier between conformers A and B. This modification of the conformational mobility could result in a perturbation of the calcium recognition properties and its transport. Further experimental investigations on this interesting point required the synthesis of the unknown modified molecule which was not straightforward. We thus decided to focus on the 19-demethylcalcimycin preparation.

As the bacterial ionophore was available in our laboratory we chose a hemisynthetic approach that would supply a large enough sample rather than a multistep total synthesis.[9] This project was supported by recent progress made in the catalysed retrograde-Aldol reaction. In our strategy, the central point was the open chain intermediate shown in Fig. 3, allowing an appropriate cleavage of the C_{18}–C_{19} bond of the released β-hydroxyketone.

Modification of the calcimycin skeleton depicted in Scheme 1 was undertaken on the known methyl ester **1**[9] obtained quantitatively by a classical treatment of the bacterial metabolite (CH_2N_2 in ether). The spiroketal moiety proved to be very

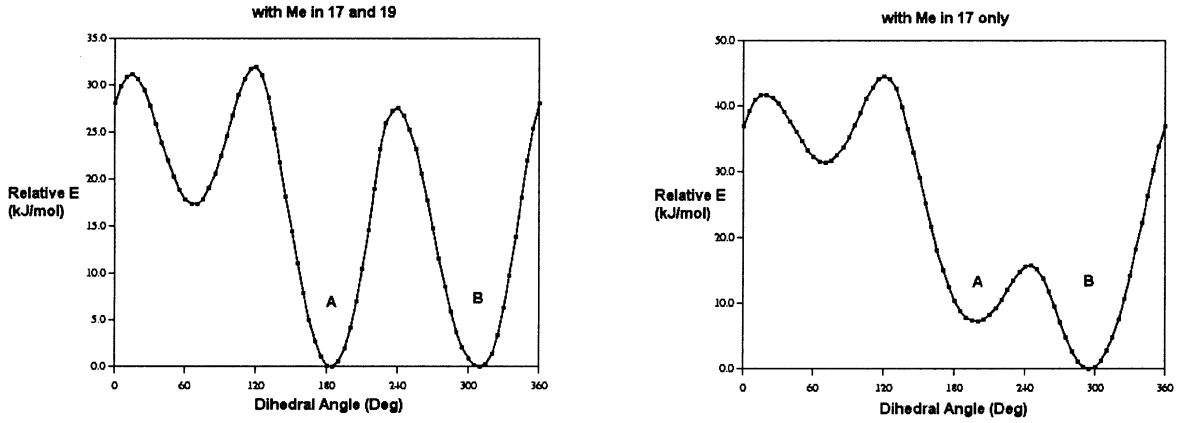

Fig. 2 Energy plots versus torsion angle for the dihedral angle C17–C18–C19–C20.

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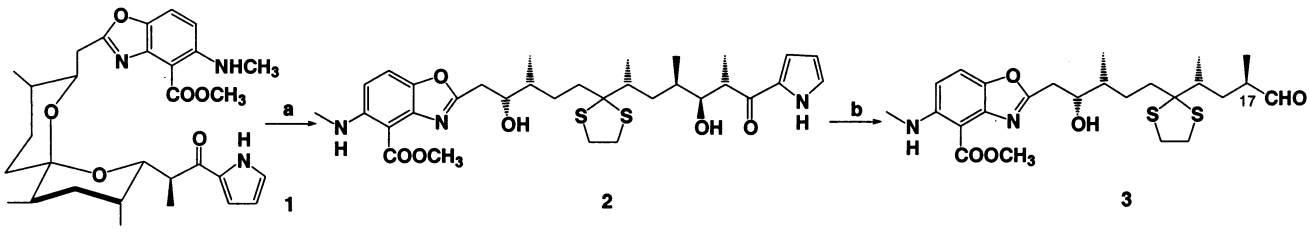
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



Scheme 1
(a) $BF_3.Et_2O$, 100 eq., $(CH_2SH)_2$, –40°C→0°C, 87%; (b) $Ti(OtBu)_4$, C_6H_5-CHO, $CHCl_3$, reflux, 45%.

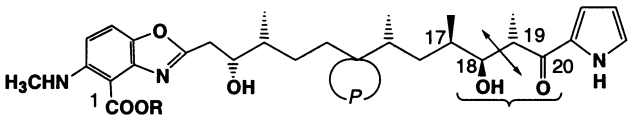


Fig. 3 Postulated open chain intermediate and C18–C19 cleavage. *P* is a protective group for the ketone function.

stable, and the open structure **2** (step a) was obtained in high yield, following previous work on model systems,[10,11] only by using drastic conditions with a large excess of $BF_3.Et_2O$ in ethanedithiol as solvent. Cleavage of the C_{18}–C_{19} bond (step b) was expected with a minimal epimerisation on the C_{17}(*R*) atom. As shown in Table 1, NaOMe, tried first, gave interesting yield but afforded too much (*S*) epimer. A recent retroaldolisation study conducted on rapamycin[12] and mediated by the coupled reagents $Ti(OiPr)_4$ / benzaldehyde prompted us to develop a corresponding approach. Finally $Ti(OtBu)_4$ / benzaldehyde was selected as additional transesterification on the carboxyl ester group, which was very efficient with $Ti(OiPr)_4$, did not occur with this hindered homologue, and the epimerisation ratio was slightly better for a similar fair yield.

Table 1 Experimental conditions tested for step b

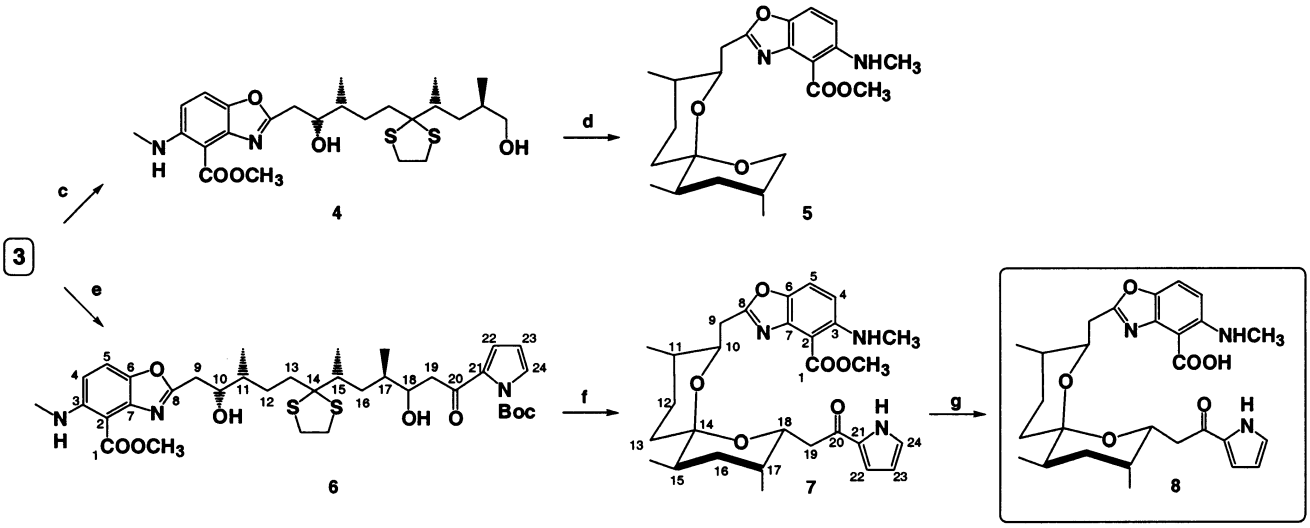
Step b Solvent	Temp.	Reagent	Yield	%17*R*/17*S*
MeOH	40°C	NaOMe 0,7M	75%	66 / 34
CH_2Cl_2	Reflux	$Ti(OiPr)_4$*	15%	86 / 14
C_6H_6	Reflux	$Ti(OiPr)_4$*	17%	
$CHCl_3$	Reflux	$Ti(OiPr)_4$*	45%	
$CHCl_3$	Reflux	$Ti(OtBu)_4$*	45%	92 / 8

* 12 equiv. Ti complex and 1.2 equiv. benzaldehyde

DIBAH reduction of calcimycin fragment **3** (step c, compound **4**) and spirocyclisation (step d), gave access to compound **5**, a calcimycin fragment that has lost its ketopyrrole arm (Scheme 2). This structure, with an axial 17-CH_3 group, confirmed the (*R*) configuration of the major isomer in epimers **3**.

Introduction of the ketopyrrole arm (step e) *via* 2-acetylpyrrole-*N*-Boc (Scheme 2) was accomplished in high yield following Kishi's aldolisation procedure[13] used in the calcimycin total synthesis. This was expected to give primarily the *syn* Cram addition product.[9] However, in our case the diastereoselectivity was not greater than 3:2.[14] The reaction mixture was isolated without diastereomers separation. Step f was a one-pot reaction with deprotection of the thioacetal[15] and carbamate groups followed by spirocyclisation with an acidic resin and molecular sieves as used for **5**. At this stage, the diastereomers were separated by chromatography furnishing the expected spiroketal compound. The stereochemistry of the C_{14} spirocenter with axial-axial C–O junction and equatorial functionalised arms in 10 and 18 positions, was confirmed on the basis of coupling constants, NOE interactions (H10*ax*–H18*ax* and H16*ax*–H18*ax*) and by comparison with the methyl ester **1**. The targeted 19-demethylcalcimycin **8** was finally obtained from the methyl ester using LiSPr in DMPU (step g).

In conclusion, we have developed a totally new hemisynthetic approach to obtain 19-demethylcalcimycin in five steps from the methyl ester of the bacterial metabolite. This approach *via* the open chain fragment **3** offers many possibilities for obtaining new structures with potentially interesting biological activities.



Scheme 2
(c) DiBAH 1M/hexane, CH_2Cl_2, rt, 72%; (d) $HgCl_2$, $CaCO_3$, CH_3CN/H_2O, rt, then Amberlyst® 15 with molecular sieves 4Å, 60%. (e) $(C_6H_{11})_2NMgBr$, THF, – 78°C, then 2-acetylpyrrole-*N*-Boc, 92%; (f) $HgCl_2$, $CaCO_3$, CH_3CN/H_20, rt, then Amberlyst® 15 with molecular sieves 4Å, 60%; (g) LiS-*n*-Pr, DMPU, rt, 92%.

Experimental

Calcimycin (or A23187) was from the stock sample of our laboratory. All the reactions were performed under argon in light protected glassware with dry solvents. Compounds were purified by flash chromatography on silica gel (Geduran SI 60, Merck, 0,040–0,063 nm). They were characterised by a complete analysis of their NMR spectra (^{1}H NMR ($CDCl_3$, 400 MHz); ^{13}C NMR ($CDCl_3$, 100 MHz)) (Bruker AC400), Low-resolution MS (EI and IC) for the main fragmentations (Hewlett Packard 5989B of our laboratory) and high resolution MS (LSIMS with Cs^+, positive mode in *m*-nitrobenzyl alcohol; MS/MS ZABSpec TOF micromass of the CRMPO, Université de Rennes 1).

Procedure for the synthesis of key compounds **3, 6** and **7**:

3 : To a solution of β-hydroxyketone **2** (2.0 g, 3.1 mmol) in $CHCl_3$ (120 ml) were added simultaneously benzaldehyde (380 µl, 3.8 mmol) and $Ti(OtBu)_4$ (14 ml, 36.6 mmol) at room temperature. The mixture was warmed to reflux for 9 h. After cooling to ambient temperature, the solution was half-concentrated then diluted with AcOEt (15 ml) and acidified with 1M HCl (10 ml). The aqueous layer was extracted with AcOEt (3 × 15 ml). The combined organic phases were washed with saturated aqueous NaCl (2 × 10 ml), dried ($MgSO_4$), filtered and concentrated under vacuum. The crude product was purified by flash chromatography ($CHCl_3$-acetone 30 : 1 → 20 : 1) to afford a diastereomeric mixture of **3** (17R/17S = 23/2) (673 mg, 45%) as yellow crystals.

6 : To a stirred suspension of Mg ribbon (250 mg, 10.3 mmol) in THF (5 ml) was added EtBr (740 µL, 10.0 mmol) at room temperature. When all of the Mg was consumed, the solution was warmed to 40°C before introducing dicyclohexylamine (2.1 ml, 10.5 mmol) in THF (10 ml) over a 5-min period. The solution was refluxed for 30 min.

To a cooled (–50°C) solution of acetylpyrrole-*N*-Boc (560 mg, 2.7 mmol) in THF (27 ml) was added the freshly prepared Grignard reagent (4.2 ml, 2.4 mmol). The mixture was stirred for 30 min at –50°C and then allowed to warm to 0°C. Aldehyde **3** (402 mg, 0.8 mmol) in THF (4.2 ml) was introduced and the reaction was maintained at 0°C for 20 min, quenched by addition of saturated aqueous NH_4Cl (14 ml), and followed by dilution with Et_2O (80 ml). The aqueous layer was extracted with 3 : 1 Et_2O/CH_2Cl_2 (2 × 60 ml). The combined organic extracts were washed with saturated aqueous NaCl (60 ml) and dried ($MgSO_4$). After filtration and concentration under reduced pressure, the residue was purified by flash chromatography ($CHCl_3$-acetone 25 / 1) providing **6** (520 mg, 92%) as yellow crystals.

7 : To a solution of **6** (420 mg, 0.6 mmol) in 25 ml of 4 : 1 CH_3CN/H_2O was introduced successively $CaCO_3$ (673 mg, 6.7 mmol) and $HgCl_2$ (1.51 g, 5.6 mmol). The mixture was stirred for 12 h at room temperature, diluted with CH_2Cl_2 (90 ml) and filtrated through a pad of Celite® followed by elution with CH_2Cl_2 (800 ml). The filtrate was successively washed with 5M aqueous NH_4OAc (210 ml), water (210 ml) and saturated aqueous NaCl (210 ml). The organic layer was dried (Na_2SO_4), filtered and evaporated *in vacuo*.

The crude residue (385 mg) was dissolved in THF (7.5 ml), acidic resin Amberlyst® 15 (500 mg) and 4Å molecular sieves were added. The mixture was stirred at ambient temperature for 14 h. After filtration, the resin was washed with AcOEt (75 ml) and the filtrate was concentrated under reduced pressure to give a residue (253 mg) which was used directly in the next step without further purification.

A solution of this crude material in CH_2Cl_2 (50 ml) was treated by TFA (5 ml). After 2.5 h at room temperature, the solution was diluted with AcOEt (250 ml), washed with 10% aqueous NaOH (100 ml), and water (75 ml). The organic layer was dried (Na_2SO_4), filtered and concentrated. Then, a careful purification by flash chromatography ($CHCl_3$-acetone 40 / 1) afforded the expected 19-demethylcalcimycin ester **7** (160 mg, 51%, one-pot from **6**) as a yellow crystals.

Spectral data for key compounds and target acid **8** :

3 : ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.65 (s, 0.92H, H18), 9.57 (d, *J*=3.0, 0.08H, H18), 8.13–7.65 (m, 1H, NHamino), 7.50 (d, *J*=9.0, 1H, H5), 6.63 (d, *J*=9.0, 1H, H), 4.22–4.11 (m, 1H, H10), 3.96 (s, 3H, CH_3 ester), 3.29–3.17 (m, 4H, CH_2-S), 3.11–2.96 (m, 2H, H9), 2.94 (bs, 3H, CH_3 amino), 2.52–2.31 (m, 1H, H17), 2.11–1.71 (m, 6H, H), 1.71–1.61 (m, 1H, H), 1.59–1.42 (m, 1H, H), 1.39–1.16 (m, 2H, H17), 1.10 (d, *J*=7.0, 6H, H15', H11'), 1.02 (d, *J*=7.5, 3H, H17'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 205.4 (C18-min), 204.8 (C18-maj), 168.7 (C1), 166.6 (C8), 150.9 (C3), 142.1 (C7), 141.3 (C6), 116.4 (C5), 108.3 (C4), 100.2 (C2), 77.7 (C14), 71.9 (C10), 51.8 (CH_3 ester), 44.9 (C17), 41.4 (C15), 40.9 (C15), 40.2 (C-S), 40.0 (C13), 39.9 (C13), 38.6 (C17), 35.7 (C16), 33.9 (C16), 33.2 (C9), 30.3 (CH_3 amino), 29.4 (C12), 29.3 (C12), 17.1 (C17'), 16.6 (C17'), 15.2 (C15'), 14.7 (C15'), 12.9 (C11'). MS (EI): *m/z* 509 (M^++1), 409, 220, 189, 131. HR-LSIMS (m-NBA, Cs^+) *m/z* M^+ : calcd. For $C_{25}H_{36}N_2O_5S_2$, 508.2066 ; found, 508.2072 $(M+H)^+$: calcd. For $C_{25}H_{37}N_2O_5S_2$, 509.2144 ; found, 509.2149.

6 : ^{1}H NMR($CDCl_3$, 400 MHz): δ (ppm) 9.73 (s, 1H, NH pyrrole), 7.87 (q, *J*=4.0, 1H, NHamino), 7.52 (d, *J*=9.0, 1H, H5), 7.08–7.00 (m, 1H, H22), 7.00–6.95 (m, 1H, H24), 6.65 (d, *J*=9.0, 1H, H4), 6.31–6.26 (m, 1H, H23), 4.20–4.12 (m, 1H, H10), 3.97 (se, 1H, OH), 3.90 (s, 3H, CH_3 ester), 3.64–3.57 (m, 1H, H18), 3.38–3.27 (m, 3H, H19+OH), 3.25–3.12 (m, 4H, CH_2-S), 3.10–2.95 (m, 2H, H9), 2.93 (d, *J*=4.0, 3H, CH_3 amino), 1.03 (d, *J*=6.5, 3H, H11'), 0.95 (d, *J*=6.0, 6H, H15', H17'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 195.3 (C20), 168.9 (C1), 166.6 (C8), 151.0 (C3), 142,0 (C7), 141,3 (C6), 131.7 (C21), 126.0 (C24), 117.5 (C22), 116.3 (C5), 110.9 (C23), 108.2 (C4), 100.2 (C2), 79.4 (C18), 78.1 (C14), 71.8 (C10), 51.7 (CH_3 ester), 41.1 (C15), 40.1 (C-S), 39.7 (C-S), 39.7 (C13), 39.2 (C19), 38.7 (C11), 37.9 (C16), 35.0 (C17), 33.4 (C9), 30.2 (CH_3 amino), 29.3 (C12), 16.6 (C17'), 14.6 (C15'), 13.5 (C11'). HR-LSIMS (*m*-NBA, Cs^+) *m/z* M^+ : calcd. For $C_{25}H_{36}N_2O_5S_2$, 717.3118 ; found, 717.3134.

7 : ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.10 (s, 1H, NH pyrrole), 7.84 (m, 1H, NHamino), 7.53 (d, *J*=9.0, 1H, H5), 6.93–6.80 (m, 2H, H22, H24), 6.60 (d, *J*=9.0, 1H, H4), 6.21–6.11 (m, 1H, H23), 4.15–4.03 (m, 2H, H10, H18), 3.95 (s, 3H, CH_3 ester), 3.01 (dd, *J*=14.5, 8.5, 1H, H9A), 2.99 (dd, *J*=13.5, 8.5, 1H, H19A), 2.92 (bs, 3H, CH_3 amino), 2.85 (dd, *J*=14.5, 6.5, 1H, H9B), 2.55 (dd, *J*=13.5, 4.5, 1H, H19B), 1.85–1.55 (m, 5H), 1.50–1.36 (m, 1H), 1.30–1.10 (m, 4H), 0.95 (d, *J*=7.0, 3H, H15'), 0.93 (d, *J*=7.0, 3H, H11'), 0.87 (d, *J*=6.0, 3H, H17'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 189.6 (C20), 169.0 (C1), 166.0 (C8), 150.8 (C3), 142.5 (C7), 142.1 (C6), 132.8 (C21), 124.2 (C24), 116.7 (C22), 116.5 (C5), 110.3 (C23), 108.0 (C4), 100.4 (C2), 99.0 (C14), 69.0 (C18), 68.7 (C10), 51.9 (CH_3 ester), 42.3 (C19), 35.3 (C16), 32.7 (C9), 32.4 (C15), 31.7 (CH_3 amino), 30.4 (C11), 29.3 (C17), 26.0 (C12), 25.7 (C13), 16.2 (C15'), 11.9 (C11'), 10.8 (C17'). HR-LSIMS (*m*-NBA, Cs^+) *m/z* M^+ : calcd. For $C_{29}H_{37}N_3O_6$, 523.2682 ; found, 523.2667. $(M+H)^+$: calcd. For $C_{29}H_{38}N_3O_6$, 524.2761 ; found, 524.2758.

8 : $[\alpha]^{25}_D$ -38 (*c* 2%, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.91 (bs, 1H, NH pyrrole), 8.73 (bs, 1H, NHamino), 7.53 (d, *J*=9.0, 1H, H5), 7.02 (bs, 1H, H22), 6.90 (bs, 1H, H24), 6.61 (d, *J*=9.0, 1H, H4), 6.27–6.19 (m, 1H, H23), 4.40 (td, *J*=7.0, 2.0, 1H, H10), 4.20 (td, *J*=3.0, 9.0, 1H, H18), 3.16–2.80 (m, 4H), 2.95 (bs, 3H, CH_3 amino), 2.03–1.06 (m, 9H), 0.96 (d, *J*=7.0, 3H, H15'), 0.94 (d, *J*=6.0, H11'), 0.88 (d, *J*=6.0, 3H, H17'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 188.7 (C20), 168.4 (C1), 166.0 (C8), 150.8 (C3), 141.6 (C7), 140.8 (C6), 132.7 (C21), 124.7 (C24), 116.9 (C22), 116.6 (C5), 110.2 (C23), 108.5 (C4), 98.8 (C2), 97.9 (C14), 69.6 (C10), 67.5 (C18), 41.5 (C19), 35.2 (C16), 32.4 (C9), 32.2 (C15), 31.5 (C11), 30.0 (CH_3 amino), 29.0 (C17), 25.9 (C12), 25.5 (C13), 16.2 (C15'), 12.2 (C11'), 10.9 (C17'). HR-LSIMS (*m*-NBA, Cs^+) *m/z* $(M+Na)^+$: calcd. For $C_{28}H_{35}N_3O_6Na$, 532.2424 ; found, 532.2412. $(M+K)^+$: calcd. For $C_{28}H_{35}N_3O_6K$, 548.2163 ; found, 548.2160.

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8 MacroModel® Program was the commercial version 7.0. Interatomic distances and angles were those of ref. 5. We were interested in this investigation by the ketopyrrole arm mobility governed by interactions in the 17, 18, 19, 20 part of the molecule, with or without a 19-Me group. Thus, in the model used for calculations, the mobile benzoxazole arm was replaced by a methyl group in 10*eq* position. OPLS-AA force field was used with conjugate gradient energy minimization to obtain the torsion angle versus energy plots represented in Fig. 2.
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