Tetrahedron Vol. 46, No. 15, pp. 5177-5186, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

SEMI-SYNTHESIS OF A.23187 (CALCIMYCIN) ANALOGS WITH 5-N-AMINO SUBSTITUENTS. THEIR COMPLEXATION OF CALCIUM AND MAGNESIUM .

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(*Received in France* 12 *April* 1990)

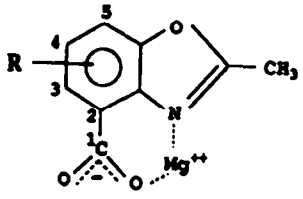
ABSTRACT : The semi-synthesis of A.23187 (calcimycin) analogs **1**~**4** bearing the respective substituents : 5-NH_2, 5-$NHCH_3$, 5-$N(CH_3)_2$, 5-$NHCOCH_3$ was carried out. The complexing properties of **1**~**4** for calcium and magnesium were determined by two-phase experiments (water/toluene-butanol 70:30 W/W) and found to be much weaker than these of A.23187 or X.14885A. There was a discrepancy with theoretical predictions made from model carboxybenzoxazoles.

INTRODUCTION

A.23187 or Calcimycin is a carboxylic polyether antibiotic isolated from a strain of *Streptomyces chartreusis*[1]. Owing to its ability to transport calcium through membranes, this ionophore is now largely used as a tool for investigating the role of this ion, as a second messenger in biology. Thermodynamic[2-5] and kinetic studies[6,7] of its associations with alkaline and/ or alkaline-earth cations were carried out in methanol and methanol-water media. X.14885A, a closely related microbial analog of A.23187 was recently studied by the same physico-chemical investigations[8].

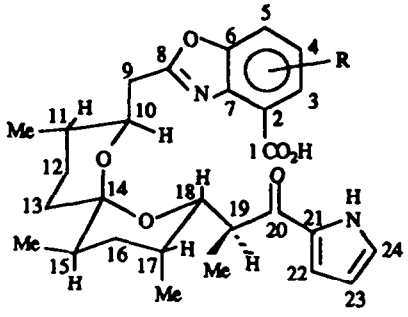
For A.23187, crystallographic determinations have shown that the benzoxazole ring provides two coordinating sites in the 2:1 complexes studied with calcium[9] and magnesium[10]. We have developed a semi-synthetic method for the preparation of analogs, from a cleaved calcimycin[11,12], with modified benzoxazole moiety, to study the role of the substituents in the complexation-decomplexation process. Theoretical calculations carried out independently on models[13] showed that Mg^{2+} binding energies on the carboxylate-benzoxazole moiety were very sensitive to the nature of the substituent R (Scheme 1).

Scheme 1



Among the different substituents studied, a maximum enhancement of the cation affinity was predicted with R = 5-N$(CH_3)_2$[13]. Thus, we undertook the preparation of the corresponding A.23187 analog 3. The semi-synthetic scheme used provided us with three other related 5-N substituted compounds 1, 2 and 4 (Scheme 2). A comparison of the complexing properties of 1-4 for calcium and magnesium, with A.23187 and X.14885A, was then carried out by two phase experiments (water/toluene-butanol 70:30 W/W) to seek confirmation of the theoretical predictions made in the gas phase on a highly simplified model.

Scheme 2

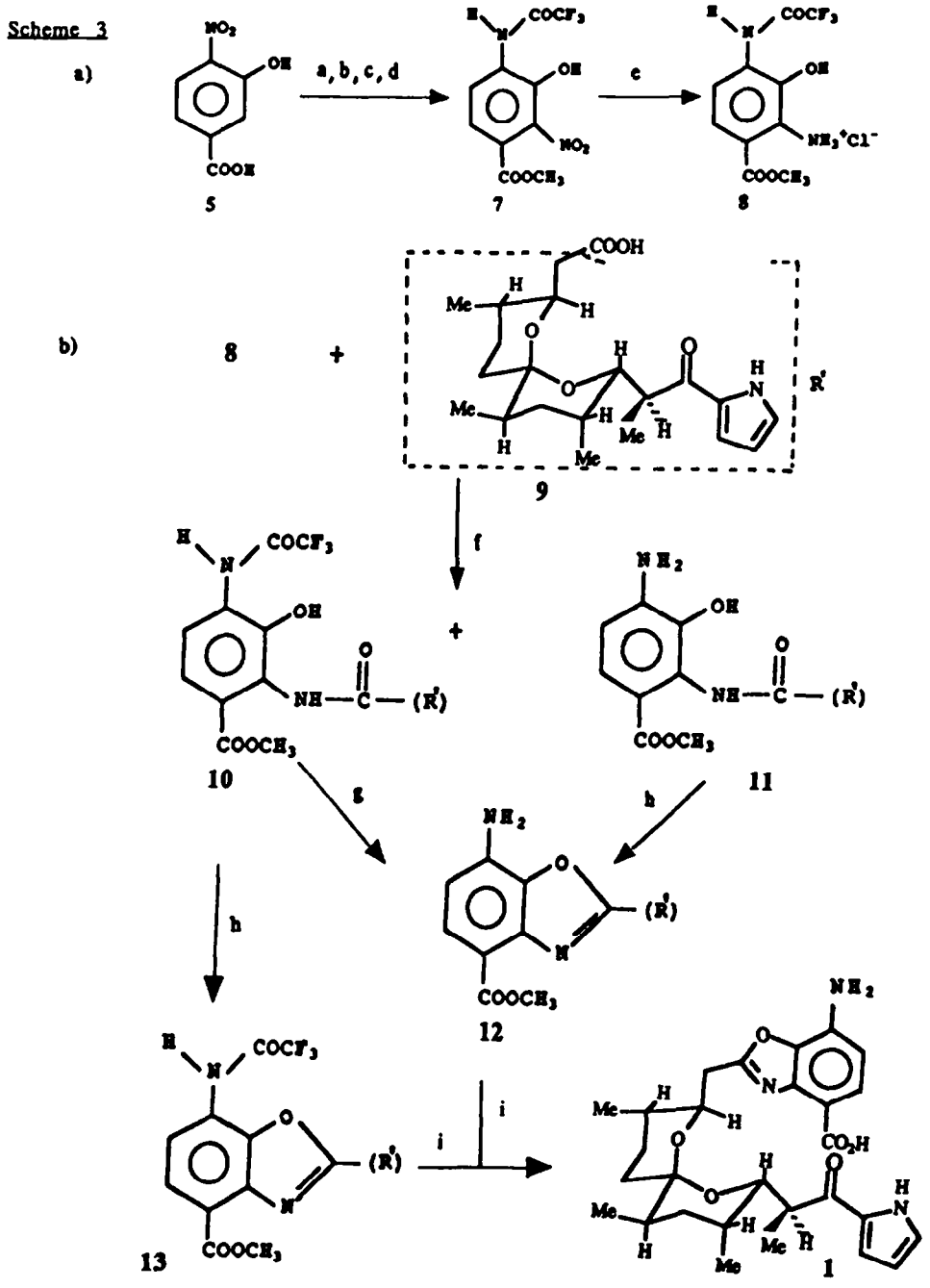


Compounds	R
A.23187	3-NHCH$_3$
X.14885A	3-OH (15-Me missing)
1	5-NH$_2$
2	5-NHCH$_3$
3	5-N$(CH_3)_2$
4	5-NHCOCH$_3$

SEMI-SYNTHESIS

Our preparations required as precursor a 3-hydroxy anthranilic ester bearing a 5-N amino substituent. We encountered difficulties in working with the unstable aromatic amines and thus chose amides as protected amine forms. Key ester 8 was synthesized from the commercial compound 5 (Scheme 3a). Attempts to obtain 7 by conventional nitration[14] (ether-fuming nitric acid solution) gave a mixture of 3-nitro, 7-nitro and 3,7-dinitro derivatives in which 7 was isolated with a very poor yield. A selective nitration was achieved using catalytic amounts of $La(NO_3)_3,6H_2O$[15] isolated yield of 7 obtained as the unique product was 59%; the unreacted material could be recycled. Finally, compound 8 was obtained as its chlorhydrate. It was then coupled with the cleaved calcimycin 9[11] (Scheme 3b) by using Benzotriazol-1 yloxy-tris-(dimethylamino) phosphonium hexafluorophosphate (or BOP)[16] to yield mainly the two products 10 (63 %) and 11 (11 %), when stoichiometric amounts of 8 and synthon 9 were used (other minor products were identified but are not described here).

Scheme 3



(a) $MeOH/H_2SO_4$: **5a**. (b) H_2, Pd/C [ethanol] : **5b**. (c) TFAA, pyridine [ether] : **6**. (d) $La(NO_3)_3,6H_2O$; $NaNO_3$ [ether]; H_2O, HCl : **7**. (e) H_2, Pd/C [ethanol]; HCl gas [ether] : **8**. (f) BOP, Et_3N, 50°C [DMF] : **10**, **11**. (g) EPP, reflux [$CHCl_3$] : **12**. (h) PPTS, 85°C [CH_2Cl-CH_2Cl] : **12**, **13**. (i) KOH 10% [ethanol] : **1**.

Cyclisation into oxazoles gave different results according to the reagents used. Compound **12** was obtained either from **10** with ethyl polyphosphate (EPP) in refluxed chloroform, with loss of the trifluoacetyl protection, or from **11** with pyridinium p-toluenesulfonate (PPTS) in dichloroethane. The use of PPTS provided **13** directly from **10**. The hydrolysis of the ester group gave the calcimycin analog **1** both from **12** and **13**, the trifluoroacetyl group being once again cleaved. The analog **4** was obtained from **1** by a conventional reaction with acetic anhydride in pyridine. Methylation of the amide **13** carried out with methyl iodide in acetone, gave a mixture of unreacted **13** and the methylated product **14** which could not be separated. However, the hydrolysis of the mixture afforded **2** with 67 % yield and **1**. Finally, the analog **3** was prepared from **2** by reaction with methyl iodide in methanol, in the presence of potassium hydroxyde.

IONIC EXCHANGES IN A TWO-PHASE SYSTEM

In the commonly accepted transport mechanism of a divalent cations M^{2+} by calcimycin[17], the carboxylic ionophore AH is deprotonated at the membrane/water interface to form a 1:1 complex (AM^+) with M^{2+}, the binding of a second A^- species creates a neutral dimeric complex A_2M which can diffuse through the membrane and is dissociated at the second interface.

This multistep process can be modeled in a two-phase system such as water/toluene-butanol (70:30)[18], where the affinity of an ionophore present in the organic phase for a divalent cation in the aqueous phase can be measured. We have already used this method[12]. Extraction curves thus obtained for A.23187, X.14885A and analogs **1**-**4** prepared are shown on figure 1 for Ca^{2+} and figure 2 for Mg^{2+}. The calcium extraction curve for compound **4** (5-$NHCOCH_3$) is drawn separately (figure 3) to show the discontinuity due to the hydrolysis of the amide function occurring for basic pH. In this region, the curve is then identical to that of compound **1** (5-NH_2).

The overall exchange equilibrium studied can be written :

$$2\ AH_{org.} + M^{2+}_{aq.} \overset{\beta_1}{\rightleftharpoons} A_2M_{org.} + 2H^+_{aq.} \quad (1)$$

which includes the acid dissociation (2) and the complex formation (3):

$$AH_{org.} \overset{K_a}{\rightleftharpoons} A^-_{aq.} + H^+_{aq.} \quad (2)$$

$$2\ A^-_{aq.} + M^{2+}_{aq.} \overset{\beta_2}{\rightleftharpoons} A_2M_{org.} \quad (3)$$

The formation of the neutral 2:1 complex for a ligand is better depicted by the equilibrium (3) which can be calculated from (1), but it is necessary to know K_a. However, this dissociation constant at the interface cannot be easily determined. The mixed solvent methanol-water 70:30 W/W was proposed to mimic conveniently the water/ membrane interface for this purpose[6,7]. Thus we made pKa measurements by UV spectrophotometry, according to the method of Kauffman et al.[19], in this mixed solvent. Our aim was not to obtain absolute values for β_2, but to compare closely related analogs, assuming that their pKa differences remain the same in this solvent and

Figure 1

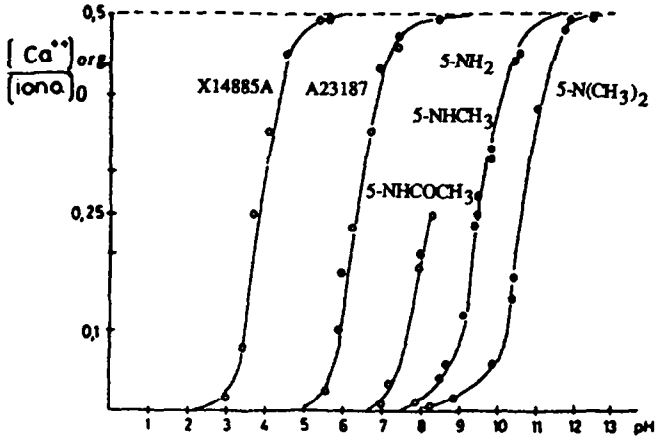


Figure 2

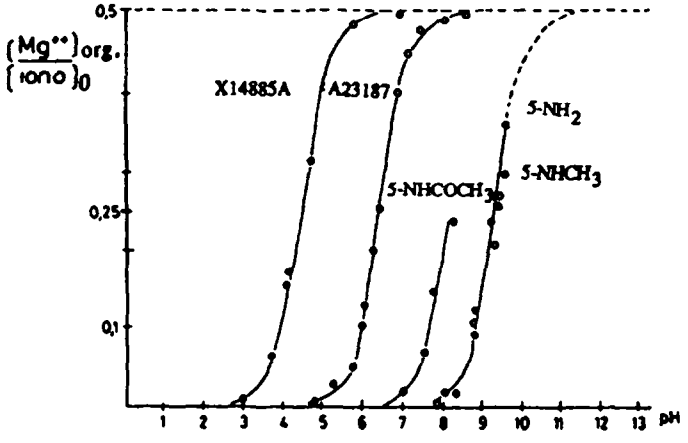
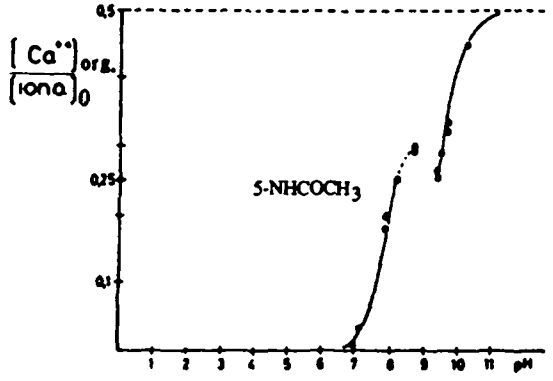


Figure 3



Effect of the aqueous phase H^+ concentration on the ionophores dependent extraction of Ca^{2+} and Mg^{2+} from the aqueous phase to the organic phase. The ionophore concentration in the organic phase was 10^{-4} M. The aqueous phase MCl_2 concentration was 10^{-2} M.

at the interface , which seems reasonable. The pK_a values obtained are given in table 1. Compound X.14885A was included in the experiment.

Table 1:

Compounds	pK_a
A.23187	7.38
X.14885A	4.48
1	6.28
2	6.40
3	8.05
*	

* pK_a determination for compound 4 was not possible because of the lability of the acetyl group at basic pH.

The extraction constants β_1 were calculated at the half-saturation points of the curves, for the ratio $[M^{2+}]_{org.}/[Ionophore]_0 = 0.25$. The values β_2 were then estimated from the expression $\beta_1 = \beta_2 \times K_a^2$ deduced from the equilibria (1)(2)(3) or $\log \beta_2 = \log \beta_1 + 2\ pK_a$. Experimental values for $\log \beta_1$ and calculated values for $\log \beta_2$ (with the pK_a values mentioned in table 1) are shown in table 2, for the two cations studied.

Table 2:

Compounds	Ca^{2+}		Mg^{2+}	
	$\log \beta_1$	$\log \beta_2$	$\log \beta_1$	$\log \beta_2$
A.23187	- 8.16	+ 6.60	- 8.26	+ 6.50
X.14885A	- 3.05	+ 5.91	- 4.36	+ 4.60
1	- 14.2	- 1.70	- 14. 3	- 1.80
2	- 14.3	- 1.55	- 14.0	- 1.26
3	- 16.8	- 0.85	*	*
4	- 11.7		-	

* The Mg^{2+} extraction with compound 3 was not studied in the same experimental conditions because the pH range required, 10.5-12, could not be reached with magnesium hydroxide owing to its insolubility.

From the values of $\log \beta_2$ obtained, one can conclude that the affinities of A.23187 and X.14885A, for calcium and magnesium, are in the same range, with a slightly better Ca^{2+}/Mg^{2+} selectivity for X.14885A, in good agreement with our results in homogeneous phase[8]. The analogs 1~4 show much weaker complexing properties in these experimental conditions.

CONCLUSION

Our experimental results did not confirm that the introduction of an electron donating substituent such as $-N(CH_3)_2$ (and $-NHCH_3$, NH_2) in 5- position stabilized the associations with calcium or magnesium, as suggested by theoretical investigations on a model[13] (Scheme 1).

This discrepancy might be explained by the difference between the simplified carboxybenzoxazole model used for the theoretical computations in the gas phase and the much more elaborate liganding systems of calcimycin and analogs, which were further studied in solution.

The benzoxazole moiety plays a central role in the capture and the release of the associated cation M^{2+}. However, if the orientation of this group is modified in analogs 1_4 during the complexation process (due to the presence of new competing liganding sites and/ or a different molecular solvation ?), this could lead to a destabilization of the AM^+ species. Further investigations are necessary to understand this dynamic process in which clearly each substituent of the benzoxazole moiety possess its own structural function to create the ionophorous property in the bacterial metabolites. We are now completing a conformational study of the compounds by NMR spectroscopy in solvents of different polarity (to be published).

EXPERIMENTAL

Melting points were determined in a Reichert hot plate microscope and are uncorrected. Thin layer chromatography (TLC) analysis was performed on Schleicher and Schüll plastic silicagel plates (F 1500/LS 254) and silicagel (Merck 60F254) glass plates for the preparative scale. Silicagel (35-70 Microns, amicon) was used for flash chromatography. Mass spectra were obtained from a Varian VG-30F spectrometer (electronic impact) or a ZABHF (FAB^+). ^1H NMR spectra were recorded on a Jeol C-60HL (60 MHz) or a Bruker MSL 300 (300 MHz) spectrometer and ^{13}C NMR spectra on a Jeol FX 60 (15 MHz) and a Bruker MSL 300 (75.47 MHz).

Methyl-3-hydroxy-4-nitrobenzoate 5a : *10 g (55 m.mol) of commercial acid 5* were dissolved in a mixture of MeOH (70 ml) and concentrated sulfuric acid (18 ml). After refluxing overnight, the solution was poured into water and extracted with ethyl acetate to give 5a as a yellow solid. Yield : 7.5 g (70 %) ; m.p. 87-88°C ; ^1H NMR (60 MHz, $CDCl_3$) δ 4.0 (s, 3H, $COOCH_3$), 7.5 (s, 1H, arom.), 7.8 and 8.4 (dd, 2H, arom.) ppm.

Methyl-5-amino-3-hydroxybenzoate 5b : 5a (6.1 g ; 31 m.mol) was dissolved in ethanol (100 ml). A catalytic amount of Pd/C was added and the mixture was hydrogenated in a Parr apparatus for 6 hours. The catalyst was filtered off on celite and the solvent removed in vacuo to give 5b as a white solid. Yield 5.1 g (98 %) ; m.p. 115°C ; ^1H NMR (60 MHz, $CDCl_3$) δ 3.8 (s, 3H, $COOCH_3$), 5.0 (broad s, 2H, NH_2), 6.8 and 7.4 (dd, 2H, arom.), 7.5 (s, 1H, arom.), 8.5 (broad s, 1H, OH) ppm.

Methyl-3-hydroxy-4-N-trifluoroacetylaminobenzoate 6 : To 5.3 g (32 m.mol) of 5b in ether (300 ml) at 0°C were added slowly over 20 minutes a mixture of ether (50 ml), trifluoroacetic anhydride (9.6 ml ; 69 m.mol) and pyridine (5.54 ml ; 68.4 m.mol). The mixture was left to progressively reach room temperature and stirred for 20 hours, then filtered. The filtrate was washed with HCl 10 % and brine. To the organic phase an equal volume of brine was added and the two-phase mixture was stirred for 45 minutes. The ether phase was dried over $MgSO_4$, the solvent removed and the residue purified by flash chromatography (eluent: cyclohexane/EtOAc 90/10) to give 6 as a white fleecy solid. Yield 5.96 g (75 %) ; m.p. 211-212°C; ^1H NMR (60 MHz, CD_3COCD_3) δ 3.9 (s, 3H, $COOCH_3$), 7.7 (s, 1H, arom), 7.9 (q, 2H arom.), 9.5 (broad s, 2H, $NHCOCF_3$, OH) ppm. ^{13}C NMR (15 MHz, CD_3COCD_3) δ 52.2 ($COOCH_3$) ; 116.3, 120.7, 122.2, 128.2, 128.7, 147.0 (arom), 166.4 ($COOCH_3$) ppm.

Methyl-3-hydroxy-2-nitro-2N-trifluoroacetylaminobenzoate 7 : $NaNO_3$ (34 mg) and La $(NO_3)_3,6H_2O$ (1.3 mg) were dissolved in a solution of H_2O (0.3 ml) and HCl (0.3 ml, d = 1.19). To this solution was added **6** (100 mg, 0.55 m.mol) in ether (2 ml) . The mixture was stirred at R.T. with T.L.C. control until the dinitro derivative began to appear, then extracted with EtOAc. The organic phase was dried over $MgSO_4$, and the residue purified by flash chromatography (eluent: cyclohexane/AcOEt 75/15) to give **7** as a yellow solid. Yield 70 mg (59 %) ; m.p. 125°C ; ^1H NMR (60 MHz, $CDCl_3$), δ 3.9 (s, 3H, $COOCH_3$), 7.9 (q, 2H, arom.), 8.6 (broad s, 1H, $NHCOCF_3$) ppm.

Methyl-2-amino-3-hydroxy-4-N-trifluoroacetylaminobenzoate 7a : Compound **7** (250 mg, 0.84 m.mol) in absolute alcool (100 ml) was reduced under hydrogen pressure with Pd/C catalyst for 2 hours. After filtration, the solvent was removed and **7a** was obtained as a white solid. Yield 224.6 mg (100 %) ; m.p. 119-120°C ; MS m/z 278 (M^+) ; ^1H NMR (300 MHz, $CDCl_3$) δ 3.9 (s, 3H, $COOCH_3$), 6.4 to 6.7 (m, 4H, 2H arom., NH_2), 7.5 (broad s, 1H, OH), 8.3 (broad s, 1H, $NHCOCF_3$) ppm.

Methyl-3-hydroxy-4-N-trifluoroacetylamino-2-N-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl)-1,7-dioxaspiro [5.5] undec-2-yl)-acetyl)-anthranilate 10 : A light protected mixture of synthon **9** (360 mg ; 0.95 m.mol) in DMF (40 ml), triethylamine (400 mg), BOP (710 mg) and **8** (300 mg ; 0.95 mmol obtained from an ether solution of **7a** by precipitation with a stream of HCl gas) was stirred in a water bath at 50°C under argon for 4 hours. After pouring into water the mixture was adjusted to pH 6, extracted with EtOAc, the organic phase was washed with water then dried over $MgSO_4$. The solvent was removed and the residue was purified by flash chromatography (eluent: $MeOH/CHCl_3$ 0.5/99.5) to give **10** as a white foam. Yield 350 mg (62.5 %) ; m.p. 85°C ; MS. m/z 638.3 $(M + H)^+$, 100 % ; exact mass calculated for $C_{31}H_{39}N_3O_8F_3$ $(M + H)^+$: 638.2679 found 638.2704. ^1H NMR (300 MHz, $CDCl_3$) δ 0.80 to 1.00 (4d, 12H, $4CH_3$), 1.00 to 1.90 (m, 8H), 2.40 (m, 1H, H_{9B}), 2.66 (m, 1H, H_{9A}), 3.20 (m, 1H, H_{19}), 3.85 (m, 1H, H_{18}), 3.95 (s, 3H, $COOCH_3$), 4.05 (m, 1H, H_{10}), 6.25 (m, 1H, H_{23}), 7.05 (m, 1H, H_{24}), 6.95 (m, 1H, H_{22}), 8.47 (d, 1H, arom.), 7.74 (d, 1H, arom.), 8.81 (s, 1H, OH), 9.25 (s, 1H, NHCOR), 9.65 (s, 1H, NH pyrrole), 12.50 (s, 1H, $NHCOCF_3$) ppm and **11** as a white foam. Yield 57 mg (11 %) m.p. 95°C ; SM. m/z 542 $(M + H)^+$ 100 %, exact mass calculated for $C_{29}H_{39}N_3O_7$ $(M + H)^+$: 542.2428 found 542.2856. ^1H NMR (300 MHz, $CDCl_3$) δ 0.80 to 1.00 (4d, 12H, $4CH_3$), 1.00 to 1.90 (m, 8H), 2.50 (m, 1H, H_{9B}), 2.70 (m, 1H, H_{9A}), 3.20 (m, 1H, H_{19}), 3.95 (m, 1H, H_{18}), 3.85 (s, 3H, $COOCH_3$), 4.10 (m, 1H, H_{10}), 6.28 (m, 1H, H_{23}), 7.00 (m, 2H, H_{24} and H_{22}), 7.40 (d, 1H, arom.), 6.50 (d, 1H, arom.), 9.25 (s, 1H, OH), 9.48 (s, 1H, NHCOR), 9.25 (s, 1H, NH pyrrole) ppm.

Methyl-7-amino-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl)-1,7-dioxaspiro [5.5] undec-2-yl)-methyl)-4-benzoxazolecarboxylate 12 :
From **10** : A light protected solution of **10** (134 mg, 0.21 mmol), ethyl polyphosphate (EPP 2.6 mmol) and $CHCl_3$ (10 ml) was refluxed for 4 hours under argon. After pouring into water and extraction with EtOAc, the organic phase was dried over $MgSO_4$ and the solvent was removed. The resulting oil was purified by T.L.C. (eluent: cyclohexane/AcOEt 50/50) to give **12** as a white foam. Yield 40 mg (30.5 %) ; m.p. 75°C ; MS. m/z 524 $(M + H)^+$:100 %, exact mass calculated for $C_{29}H_{39}N_3O_6$ $(M + H)^+$: 524.2751 found 524.2534. ^1H NMR (300 MHz, $CDCl_3$) δ 0.70 to 1.10 (4d, 12H, $4CH_3$), 1.10 to 1.80 (m, 8H), 2.85 (m, 1H, H_{9B}), 3.10 (m, 1H, H_{9A}), 3.25 (m, 1H, H_{19}), 3.52 (m, 1H, H_{18}), 3.91 (s, 3H, $COOCH_3$), 4.13 (m, 1H, H_{10}), 6.28 (m, 1H, H_{23}), 7.00 (m, 1H, H_{24}), 7.03 (m, 1H, H_{22}), 7.85 and 6.95 (2d, 2H, arom.), 9.65 (s, 1H, NH pyrrole) ppm.
From **11** : A mixture of **11** (168 mg, 0.31 mmol) and pyridinium p-toluenesulfonate (PPTS, 50.5 mg, 0.20 mmol) in dicholoroethane (22 ml) were stirred overnight in an oil bath at 85°C under argon. After cooling, dilution with $CHCl_3$, the mixture was washed with a saturated solution of $NaHCO_3$, the organic phase was dried over $MgSO_4$. The solvent was removed and the residue purified by T.L.C. (eluent: cyclohexane/AcOEt 60/40) to give **12** (28 mg, 26 %).

Methyl-7-N-trifluoroacetylamino-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl)-1,7-dioxaspiro [5.5] undec-2-yl)-methyl)-4-benzoxazolecarboxylate 13 : This compound was obtained from **10** (110 mg ; 0.17 mmol) and P.P.T.S. by the method described above. The crude product was purified by T.L.C. (eluent: cyclohexane/AcOEt 60/40) to yield **13** (45 mg ; 42 %) as a white foam. m.p. 90°C ; MS. m/z 620 $(M + H)^+$ 100 % ; exact mass calculated for $C_{31}H_{36}N_3O_7F_3$ $(M + H)^+$ 620.2574 found 620.2456. ^1H NMR (300 MHz, $CDCl_3$) δ 0.70 to 1.00 (4d, 12H, $4CH_3$), 1.00 to 1.90 (m, 8H), 2.95 (m, 1H, H_{9B}), 3.15 (m, 1H, H_{9A}), 3.25 (m, 1H, H_{19}), 3.25 (s, 3H, $COOCH_3$), 3.95 (m, 1H, H_{18}), 4.05 (m, 1H, H_{10}), 6.25 (m, 1H, H_{23}), 7.05 (m, 1H, H_{24}), 6.95 (m, 1H, H_{22}), 8.05 and 7.65 (2d, 2H arom.), 10.00 (s, 1H, NH pyrrole), 11.40 (s, 1H, $NHCOCH_3$) ppm.

7-Amino-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl)-1,7-dioxaspiro [5.5.] undec-2-yl)-methyl)-4-benzoxazolecarboxylic acid 1 : A light protected mixture of **13** (73 mg ; 0.12 mmol) in ethanol (50 ml) and 10 % potassium hydroxide (2 ml) was stirred at 30°C for 4 hours, poured into water (200 ml), adjusted to pH 7 with 0.1 N HCl, extracted with EtOAc and dried over $MgSO_4$. After solvent evaporation, the residue was purified by T.L.C. (eluent: cyclohexane/AcOEt 50/50 and 15 drops of acetic acid for 100ml) to give **1** (25.2 mg ; 45.4 %) as white foam. m.p. 118°C ; M.S. m/z : 510 $(M + H)^+$ 100 % ; exact mass calculated for $C_{28}H_{35}N_3O_6$ $(M + H)^+$ 510.2595 found 510.2587. ^1H NMR (300 MHz, $CDCl_3$) δ 0.80 to 1.15 (4d, 12H, $4CH_3$), 1.15 to 1.90 (m, 8H), 2.84 (m, 1H, H_{9B}), 3.10 (m, 1H, H_{9A}), 3.28 (m, 1H, H_{19}), 3.45 (m, 1H, H_{18}), 4.18 (m, 1H, H_{10}), 6.30 (m, 1H, H_{23}), 7.08 (m, 1H, H_{24}), 7.03 (m, 1H, H_{22}), 6.98 and 7.90 (2d, 2H arom.), 10.00 (NH pyrrole) ppm.
1 was obtained from **12** by the same hydrolysis method (yield 60 %).

7-Acetylamino-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl-1,7-dioxaspiro [5.5] undec-2-yl)-methyl)-4 benzoxazolecarboxylic acid 4 : To a stirred solution of acetic anhydride (2.4 mg) in pyridine (0.4 ml) at 0°C was added the acid **1** (20 mg ; 0.04 mmol). The mixture was allowed to react for 4 hours. Most of the pyridine was removed by evaporation under reduced pressure. The residue was diluted with ether, washed with 0.1 N HCl. After solvent removal, the compound was purified by T.L.C. (eluent: AcOEt/MeOH : 99/1) to give **4** (14 mg, 66 % yield) as a white foam. m.p. 125°C ; M.S. m/z : 552 $(M + H)^+$ 100 % ; exact mass calculated for $C_{30}H_{28}N_3O_7$ $(M + H)^+$ 552.2709 found 552.2681. ^1H NMR (300 MHz, $CDCl_3$) δ 0.80 to 1.10 (4d, 12H, $4CH_3$), 1.10 to 1.90 (m, 8H), 3.12 (m, 2H, H_{9B} and H_{9A}), 3.25 (m, 1H, H_{19}), 3.75 (m, 1H, H_{18}), 4.13 (m, 1H, H_{10}), 6.29 (m, 1H, H_{23}), 7.00 (m, 2H, H_{24} and H_{22}), 7.80 and 8.20 (2d, 2H arom.), 10.78 (s, 1H, NH pyrrole) ppm.

7-N-methylamino-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl) ethyl)-1,7-dioxaspiro [5.5] undec-2-yl)-methyl)-4-benzoxazolecarboxylic acid 2 : To a solution of **13** (57 mg ; 0.09 mmol) in acetone (10 ml) were added anhydrous Na_2CO_3 (80 mg) and methyl iodide (0.2 ml, 1.2 mmol). The mixture was stirred for 3 hours at room temperature, then filtered off and evaporated to give a white foam (50mg) shown by ^1H NMR (300 MHz) to be a mixture of **14** and unreacted **13** material. The two products could not be separated. The hydrolysis was performed on the mixture with KOH in ethanol according to the method previously described. After T.L.C. purification (eluent: cyclohexane/AcOEt 40/60 and 15 drops of acetic acid for 100ml), **2** (30 mg ; 67 % yield) was obtained as a white foam. m.p. 120°C ; M.S. m/z : 524 $(M + H)^+$ 100 % ; exact mass calculated for $C_{29}H_{37}N_3O_6$ $(M + H)^+$ 524.2751 found 524.2799. ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 to 1.15 (4d, 12H, $4CH_3$), 1.15 to 1.90 (m, 8H), 3.05 (m, 1H, H_{9B}), 3.15 (m, 1H, H_{9A}), 3.30 (m, 1H, H_{19}), 3.90 (m, 1H, H_{18}), 3.37 (s, 3H, $NHCH_3$), 4.25 (m, 1H, H_{10}), 6.27 (m, 1H, H_{23}), 7.11 (m, 1H, H_{24}), 7.03 (m, 1H, H_{22}), 6.72 and 7.40 (2d, 2H arom.), 9.50 (broad s, 1H, COOH), 11.25 (s, 1H, NH pyrrole) ppm.

7-N,N-dimethylamino-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1H-pyrrol-2-yl) ethyl)-1,7-dioxaspiro [5.5] undec-2-yl)-methyl)-4-benzoxazole-carboxylic acid 3 : KOH (5.26 mg) was added to a solution of **2** (31 mg, 0.06 mmol) in methanol (0.5 ml). The mixture was stirred until it became homogeneous, cooled to 10°C, then ICH_3 (0.04 ml ; 0.24 mmol) was added and the light-protected solution stirred overnight at R.T.. After methanol removal and purification of the residue by T.L.C. (eluent: cyclohexane/AcOEt 40/60 and 15 drops of acetic acid

for 100ml) the compound 3 (15 mg, 47 % yield) was obtained as a white foam. m.p. 127°C ; M.S. m/z : 538 $(M + H)^+$ 100 % ; exact mass calculated for $C_{30}H_{39}N_3O_6$ $(M)^+$ 537.2829 found 537.2836. ^1H NMR (300 MHz, $CDCl_3$) δ 0.80 to 1.10 (4d, 12H, $4CH_3$), 1.10 to 1.90 (m, 8H), 3.12 (s, 6H, $N(CH_3)_2$), 3.18 (m, 3H, H_{9A}, H_{9B} and H_{19}), 3.50 (m, 1H, H_{18}), 4.18 (m, 1H, H_{10}), 6.27 (m, 1H, H_{23}), 6.90 (m, 1H, H_{22}), 7.00 (m, 1H, H_{24}), 7.75 and 8.31 (2d, 2H arom.), 9.50 (broad s, 1H, COOH), 9.89 (s, 1H, NH pyrrole) ppm.

The structures of compounds **10, 11, 12, 13, 1, 2, 3, 4** were confirmed by ^{13}C NMR (75 MHz) spectra which are not described here.

pKa measurements : The electronic absorption spectra were recorded on a CARY 2200 U.V.-visible spectrophotometer. The concentration of the ionophore in the methanol-water medium (70:30 W/W) was 5.10^{-5} M.

Ca^{2+} and Mg^{2+} exchange in a two phase extraction system : Equilibrium constants were determined according to the method described by Pfeiffer and Lardy[18] modified by M. Hebrant et al.[20]. The organic phase was toluene-butanol (70:30 W/W instead of V/V). Extractions were performed at 25°C. The aqueous solutions were 10^{-2} M in Ca^{2+} or Mg^{2+} and buffered with β,β'-dimethylglutaric acid/tetramethyl ammonium hydroxide (pH 3-5), HCl/bis Tris (pH 5-7), HCl/Tris (pH 7-9), HCl/dimethylethanol amine (pH 8-10.5) ; HCl/calcium hydroxide (Ca^{2+} solutions, pH 10.5-12). Ca^{2+} and Mg^{2+} amounts released after extraction by the ionophore in a HCl 0.5 N solution were measured by atomic absorption using a Perkin Elmer 420 spectrometer. Divalent cation solutions used to standardize the spectrometer were previously equilibrated with the organic phase without ionophore.

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