# Synthesis, Characterization, and Biological Evaluation as Antibacterial Agents of Water-Soluble Calix[4]arenes and Phenol Derivatives Incorporating Carboxylate Groups

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As part of specific investigations dedicated to the building of a chemical library of calixarenes with anti-infective properties, some $H_2O$-soluble calix[4]arene derivatives incorporating carboxylate groups at the upper rim and heterocycles at the lower rim have been prepared and characterized as have their constitutive functional monomers. *In vitro* antibacterial testing against *Gram*-positive and *Gram*-negative reference strains shows that the derivative with two 5,5′-dicarboxylated 2,2′-bithiazole subunits in alternate positions at the lower rim displays an activity at *ca.* 70 μM (128 μg/ml) on *S. aureus* ATCC 29213.

**Introduction.** – Faced with the emergence of strains of pathogenic microorganisms resistant to present antibiotics [1], research dedicated to the discovery of new drugs in this field has resulted in some interesting approaches involving calixarene derivatives. Nevertheless, as summarized in some recent reviews [2–5], few studies have focused on the therapeutic potential of calixarene compounds as anti-infective agents. For example, in the mid-1950s, the calixarene derivative '*macrocyclon*' [6], and, more recently, some parent structures [7–9] were studied with respect to the treatment of tuberculosis and other mycobacterioses. The building of designed calixarenic mimics of vancomycin has also been performed, with the aim of generating antimicrobial activity [10], and some biological studies related to plasmid DNA binding and cell transfection were reported by *Ungaro* and co-workers [11–13]. Certain hydrophilic derivatives have shown interesting levels of activity against bacteria, [14], fungi, cancerous cells, and enveloped viruses [15–21].

To progress in this direction, we focused on the development of new molecular devices, designed to function as hydrophobic [22] or hydrophilic [23] molecular drug dispensers. These are based on a calixarene platform with penicillin or quinolone moieties attached *via* a labile bond at the lower rim. We developed poly-ionic calixarene derivatives which display intrinsic antibacterial activity. The first derivatives of this type were designed as spatially organized and constrained tetra-guanidinium entities, in which the ionic groups were tethered at the upper rim of the calix[4]arene platform, the lower rim remaining free (OH groups) [24] or partially substituted [25]. These compounds, assumed to interact with and damage negatively charged bacterial surfaces, displayed useful antibacterial activities against various reference [24] or resistant [26] *Gram*-negative or *Gram*-positive bacterial strains. We found that these

cationic molecules have an impact on the bacterial wall [27] and should modify bacterial membrane permeability [28].

The two initial studies were carried out by a comparison of calix[4]arene derivatives with their constitutive phenolic monomers, showing the importance of pre-organization of guanidinium functions in the acquisition of antibacterial properties [24][25].

In parallel, various $H_2O$-soluble poly-anionic calixarene derivatives were evaluated as anti-HIV compounds. Among them, derivatives of the tetra-*para*-(carboxymethyl)-calix[4]arene (**1**; *cf. Scheme 1*), *i.e.*, sodium salts **11** and **13** (2,2′-bipyridyl) [29], **12** and **14** (2,2′-bithiazolyl) [30], and **15** (unsubstituted) [30] of the present study, showed interesting inhibitory activities which varied with the cell and HIV-1 strains: $IC_{50}$ values lay between 5 and 40 μM, associated with low cytotoxicity ($CC_{50} > 100$ μM).

To verify if such derivatives were also antibacterial, and if cyclooligomerization would result in the gain or acquisition of activity as found in the guanidinium family [24], we evaluated their activity against *Gram*-negative and *Gram*-positive reference strains. The study was conducted as for guanidinium analogs [24][25], with comparison of calixarene derivatives and their constitutive functional monomers.

The tetra-*para*-(carboxymethyl)calix[4]arene (**1**) has not been extensively studied in the therapeutic field; it appears essentially to be a candidate for treatment of HIV infection [15][30], or as an antithrombotic agent [31].

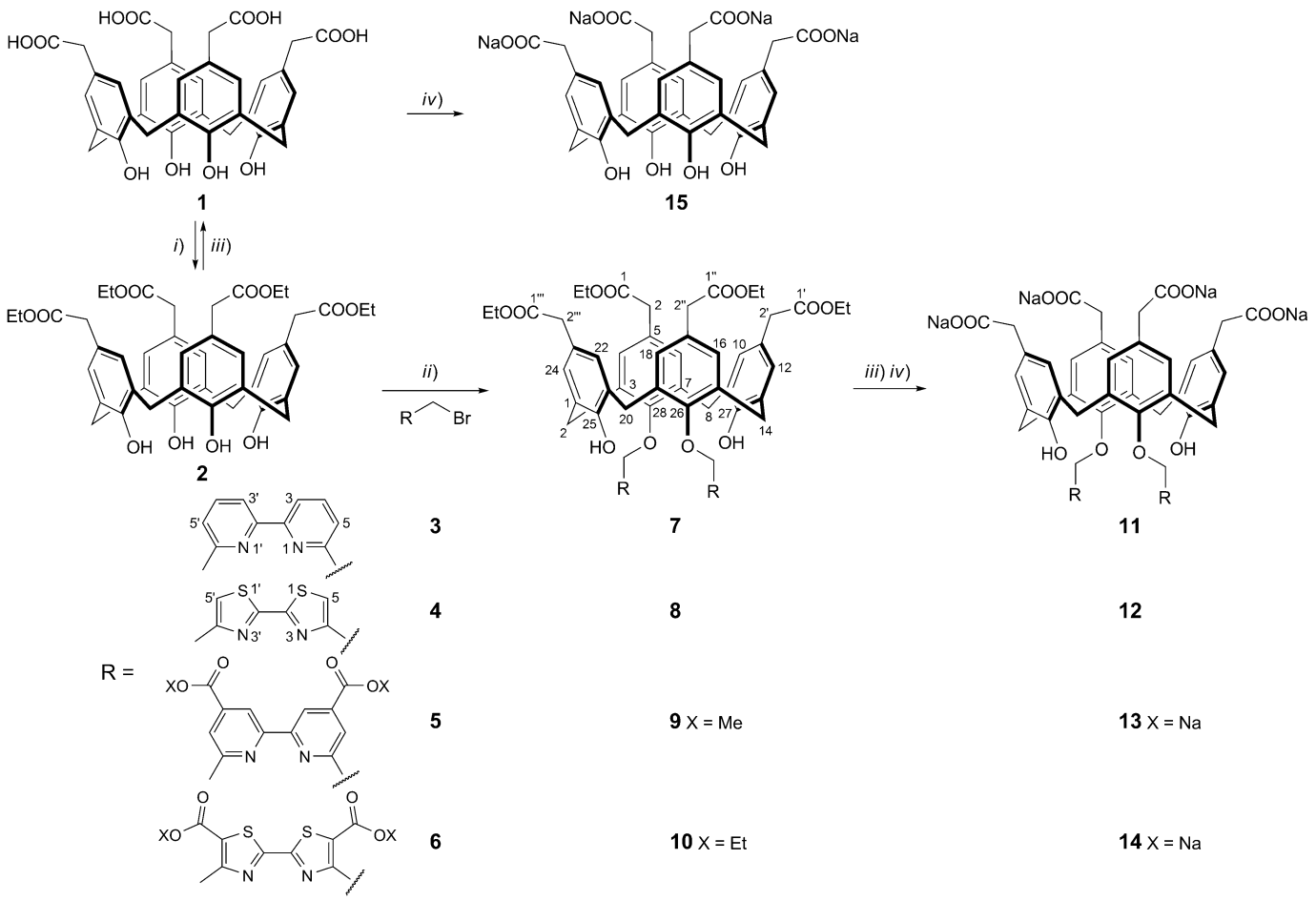
The therapeutic possibilities of 2-(4-hydroxyphenyl)acetic acid (**17**; *cf. Scheme 2*) and its derivatives have been widely studied, but few reports dealt with antimicrobial activity. *Tuncel* and *Nergiz* [32] described the absence of antibacterial activity (agar dilution technique) of **17** against *E. coli* O157:H7 (minimum inhibitory concentration (*MIC*) 600 μg/ml) and *S. aureus* NCTC 10657 S-6 ($MIC > 600$ μg/ml). In a recent patent, **17** and its ester derivatives were evaluated as conservative antibacterial agents against *A. niger*, *C. albicans*, *E. coli*, *P. aeruginosa*, and *S. aureus* (no data available) [33].

**Results and Discussion.** – 6-(Bromomethyl)-6′-methyl-2,2′-bipyridine (**3**) was prepared according to *Rodriguez-Ubis et al.* [34], its 4,4′-dimethyl ester analog **5** according to *Psychogios et al.* [35]. 4-(Bromomethyl)-4′-methyl-2,2′-bithiazole (**4**) and its 5,5′-diethyl ester analog **6** were prepared according to *Pellet-Rostaing et al.* [36], and *Lehn* and *Regnouf-de-Vains* [37], respectively. Calixarene esters, **2**, **8**, and **10**, then sodium salts **12**, **14**, and **15** were prepared by the method of *Mourer et al.* [30]. The sodium salts **11** and **13** have been previously partially described [38]. The synthetic pathways are outlined in *Scheme 1*.

The tetra acetic acid **1** and then the tetraethyl tetraacetate **2** were prepared according to the procedures of *Gutsche* and co-workers [39].

Introduction of the heterocyclic subunits in alternate positions, at the lower rim of **2** was carried out by reaction of bromomethyl derivatives **3** and **5** with **2**, in dry MeCN, in the presence of 1 equiv. of $K_2CO_3$ as base, to give, with yields of *ca.* 60% after chromatography on alumina, the corresponding tetra-ester **7** and octa-ester **9**. These were saponified with NaOH in hydro-alcoholic medium; the addition of HCl resulted in the precipitation of the corresponding tetra- and octa-acids that were carefully rinsed with $H_2O$ to remove the highest amount of residual NaCl. The acids were then carefully titrated with aq. NaOH under pH-meter monitoring, until solubilization was complete.

*Scheme 1*



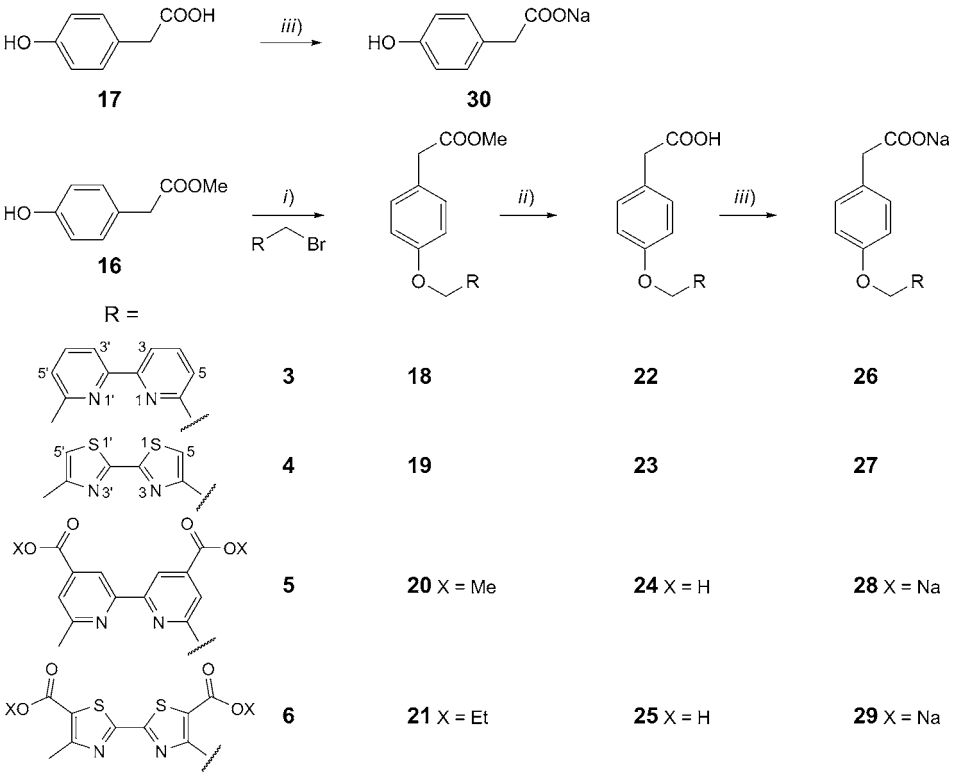

*i*) EtOH, $H_2SO_4$ 96%, reflux, 53%. *ii*) MeCN, $K_2CO_3$, reflux; **7**: 57%, **9**: 57%, (**8**: 54%, **10**: 48%; see [30]). *iii*) a) $H_2O$, EtOH, NaOH, reflux; b) HCl, $H_2O$. *iv*) 0.1M NaOH, pH-meter monitoring, dialysis (cellulose acetate, MWCO 100D); **11**: final pH 8.25, 78%; **13**: final pH 7.90, 53%; see [38]; **12**: final pH 7.10, 82%; **14**: final pH 8.0, 67%; see [30]; **15**, final pH 7.40, 74%.

The resulting solutions were dialyzed on a *Float-A-Lyser* device (cellulose acetate, molecular weight cut off (MWCO) 100 D) and lyophilized to give the tetra-sodio derivative **11**, and the octa-sodio derivative **13**, respectively.

As direct preparation of **15** from the tetra acid resulting from acidic hydrolysis of the corresponding tetra-nitrile yielded a colored compound, we decided to prepare it from the pure ester **2**, according to the above mentioned procedure.

Syntheses of monomer species was conducted as outlined in *Scheme 2*. Methyl (4-hydroxyphenyl)acetate (**16**) and the corresponding acid **17** were commercially available. The reaction of bromomethyl derivatives **3**–**6** with 1 equiv. of **16** and $K_2CO_3$ in dry MeCN afforded the esters **18**–**21**, respectively, with yields of between 50 and 97% after chromatography on alumina; the esters were then saponified in an hydro-alcoholic medium, and consecutive acidification resulted in precipitation of the corresponding acids **22**–**25**; the latter were collected, washed, then cautiously neutralized by NaOH (pH-meter monitoring), until solubilization was complete, to give, after lyophilization, the corresponding mono- or trisodium salts **26**–**29**,

*Scheme 2*




*i*) MeCN, $K_2CO_3$, reflux; **18**: 77%, **19**: 50%, **20**: 97%, **21**: 73%. *ii*) a) $H_2O$, EtOH, NaOH, reflux; b) HCl, $H_2O$; (**22**: 70%, **23**: 93%, **24**: 97%, **25**: 61%. *iii*) 0.1M NaOH, pH-meter monitoring; **26**: final pH 7.70, 96%; **27**: final pH 8.50, 94%; **28**: final pH 7.30, 79%; **29**: final pH 7.90, 82%; **30**: final pH 8.0, 94%.

respectively. The sodium salt **30** of (4-hydroxyphenyl)acetic acid was prepared from commercial acid **17** by the same procedure.

Calixarene derivatives **11** and **13** have been described previously [38], as well as have derivatives **12**, **14**, and **15** [30]. Due to their low solubility, the structures of corresponding acid derivatives were only assessed by $^1$H-NMR. All other compounds, esters and salts, were fully characterized, and gave satisfactory $^1$H- and $^{13}$C-NMR, elemental and electrospray ionization mass analyses. More particularly, $^1$H- and $^{13}$C-NMR conformational studies of the calixarene family showed that the cone conformation was preserved, with Ar–$CH_2$–Ar resonance signals appearing as *AB* systems around 3.50 and 4.35 ppm in $^1$H-NMR and as a singlet around 31 – 32 ppm in $^{13}$C-NMR (*Table 1*) [40].

Table 1. *Partial $^1$H- and $^{13}$C-NMR Data* (400 and 100 MHz, resp., in $D_2O$, r.t.) *of $H_2O$-Soluble Calixarenes* **11** – **15**. $\delta$ in ppm, $J$ in Hz.

| Ar$CH_2$Ar | $\delta$(H) | $\delta$(C) |
|---|---|---|
| **11** | 3.55, 4.38 (*AB*, $J_{AB}$ = 13.1) | 30.99 |
| **12** | 3.48, 4.35 (*AB*, $J_{AB}$ = 13.3) | 30.74 |
| **13** | 3.40, 4.19 (*AB*, $J_{AB}$ = 12.8) | 30.99 |
| **14** | 3.47, 4.40 (*AB*, $J_{AB}$ = 13.4) | 31.19 |
| **15** | 3.00, 4.18 (*AB*, $J_{AB}$ = 12.08) | 32.19 |

Initial C,H,N elemental analyses of calixarene salts **12**, **14**, and **15** suggested the presence of $H_2O$ molecules and supplementary NaCl. The latter, resulting from titration of residual free or salting HCl, was not fully eliminated during the dialysis steps. This led us, as we had already done for calixarenes **11** and **13**, to complete the elemental analyses by flame photometry. This gave results consistent with the proposed formulae (*Table 2*). Compound **12** was thus found to be associated with 1 NaCl and 6 $H_2O$, **14** with 5 NaCl and 7 $H_2O$, and **15** with 1.5 NaCl and 5.5 $H_2O$.

Table 2. *Sodium Content* [%] *of $H_2O$-Soluble Calixarenes* **11** – **15**[a])

| | Molecular formula (MW) | Na | |
|---|---|---|---|
| | | Calc. | Found |
| **11** | $C_{60}H_{48}N_4Na_4O_{12} \cdot 5.0\ H_2O \cdot 2$ NaCl (1315.90) | 10.40 | 10.16 |
| **12** | $C_{52}H_{40}N_4Na_4O_{12}S_4 \cdot 6\ H_2O \cdot$ NaCl (1299.60) | 8.84 | 8.95 |
| **13** | $C_{64}H_{44}N_4Na_8O_{20} \cdot 7.0\ H_2O \cdot 16$ NaCl (2433.94) | 22.70 | 22.40 |
| **14** | $C_{56}H_{36}N_4Na_8O_{20}S_4 \cdot 7\ H_2O \cdot 5$ NaCl (1815.24) | 16.45 | 16.08 |
| **15** | $C_{36}H_{28}Na_4O_{12} \cdot 5.5\ H_2O \cdot 1.5$ NaCl (931.21) | 13.58 | 14.03 |

[a]) Evaluated by flame photometry.

Positive-ion-mode ESI-MS analysis of calixarene derivatives **12** (*Fig. 1*) and **14** (*Fig. 2*) gave unsatisfactory results. Much better results were obtained in the negative-ion mode, following the general observations made previously with compounds **11** and **13** [38]. Compound **12** exhibited mainly dicharged species at $m/z$ 521.19, 532.16, and 543.24 a.m.u., corresponding to the loss of 4, 3, and 2 Na ions, respectively, balanced when necessary by the gain of 2 or 1 protons. At a lower intensity, monocharged species

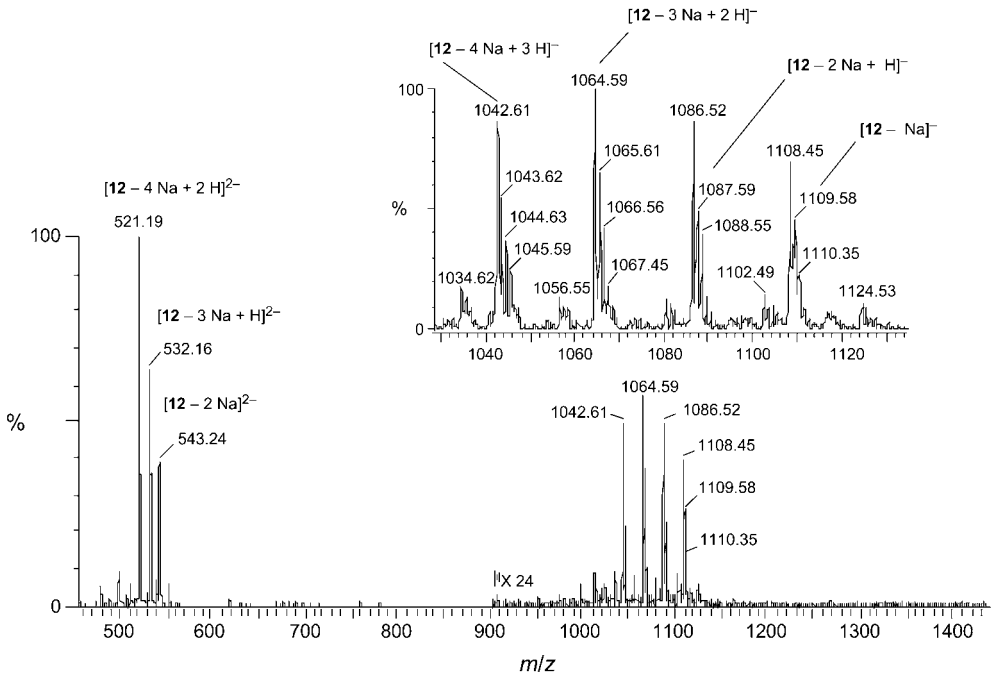



Fig. 1. *Negative-ion-mode ESI mass spectrum of calixarene* **12**

were detected at *m*/*z* 1109.58, 1086.52, 1064.59, and 1042.61 a.m.u., corresponding here to the loss of 1, 2, 3, or 4 Na ions available, respectively, balanced when necessary by protonation.

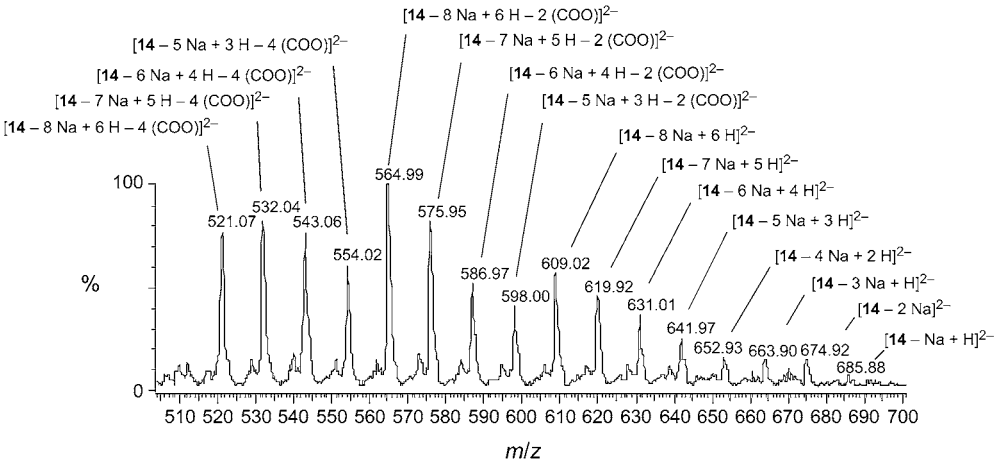



Fig. 2. *Negative-ion-mode ESI mass spectrum of calixarene* **14**

In the same mode, ESI-MS of compound **14** exhibited, in a *m*/*z* domain between 500 and 700 a.m.u., a succession of three di-charged ionic families, one resulting from the loss of 1 to 8 Na ions, one from the loss of two carboxylate groups and 5 to 8 Na ions, and one from the loss of four carboxylate groups and 5 to 8 Na ions, balanced by protonation.

All monomeric species gave analyses in accordance with the proposed formulae. The acids **22**–**25** were characterized by $^1$H-NMR and elemental analysis.

Positive-ion-mode ESI-MS analysis of monomeric salts **26**–**29** indicated the presence of only the mono-charged species, involving at least, in each case, an adduct of a Na cation.

**Biological Evaluations.** – Antibacterial activities, *i.e.*, minimum inhibitory concentrations (*MIC*), of $H_2O$-soluble calixarenes **11**–**15**, and of their corresponding monomers **26**–**30** were determined against *Gram*-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and *Gram*-positive (*Staphylococcus aureus* ATCC 25923 and ATCC 29213, and *Enterococcus faecalis* ATCC 29212) reference strains, according to microdilution protocols performed in 96-well U shape microtiter plates [41][42].

The results compiled in *Table 3* show that, except for the pair calixarene **14**/*S. aureus* ATCC 25923, none of the compounds displays any antibacterial activity under 256 μg/ml. At this stage of the study, one can suggest an effective discrimination between antiviral and antibacterial activities, in favor of the former. Compound **14** exhibits its activity at a *MIC* value of 128 μg/ml, corresponding to a 70 μM concentration; it is at least two times (mass ratio) and six times (molar ratio) more active than its monomer **29** (> 256 μg/ml, 462 μM). This could be interpreted, but to a much lesser extent, as a positive effect of cyclooligomerization, as already observed for guanidinium derivatives [24].

**Conclusions.** – We have prepared a new series of $H_2O$-soluble anionic calixarenes bearing in alternate positions at the lower rim two 2,2′-bipyridine or 2,2′-bithiazole

Table 3. *Minimum Inhibitory Concentration* (*MIC*) *Values*[a]) *in μg/ml* ([μM])

| Compound (MW) | *E. coli* ATCC 25922 | *S. aureus* ATCC 25923 | *S. aureus* ATCC 29213 | *E. faecalis* ATCC 29212 | *P. aeruginosa* ATCC 27853 |
|---|---|---|---|---|---|
| **11** (1315.9) | > 256 (> 194) | > 256 (> 194) | > 256 (> 194) | > 256 (> 194) | > 256 (> 194) |
| **26** (385.5) | > 256 (> 664) | > 256 (> 664) | > 256 (> 664) | > 256 (> 664) | > 256 (> 664) |
| **12** (1299.6) | > 256 (> 197) | > 256 (> 197) | > 256 (> 197) | > 256 (> 197) | > 256 (> 197) |
| **27** (415.6) | > 256 (> 616) | > 256 (> 616) | > 256 (> 616) | > 256 (> 616) | > 256 (> 616) |
| **13** (2433.9) | > 256 (> 105) | > 256 (> 105) | > 256 (> 105) | > 256 (> 105) | > 256 (> 105) |
| **28** (618.8) | > 256 (> 414) | > 256 (> 414) | > 256 (> 414) | > 256 (> 414) | > 256 (> 414) |
| **14** (1815.2) | > 256 (> 141) | **128** (**70.5**) | > 256 (> 141) | > 256 (> 141) | > 256 (> 141) |
| **29** (554.4) | > 256 (> 462) | > 256 (> 462) | > 256 (> 462) | > 256 (> 462) | > 256 (> 462) |
| **15** (931.2) | > 256 (> 275) | > 256 (> 275) | > 256 (> 275) | > 256 (> 275) | > 256 (> 275) |
| **30** (184.1) | > 256 (> 1390) | > 256 (> 1390) | > 256 (> 1390) | > 256 (> 1390) | > 256 (> 1390) |

[a]) Obtained by broth microdilution method, according to *NCCLS* (formerly *CLSI*) guideline (see *Exper. Part*).

subunits; their monomeric analogs were also prepared. These carboxylated species do not display clear antibacterial activities. In contrast to the previously described guanidino analogs, the cyclotetramerization of inactive 2-(4-hydroxyphenyl)acetic acid and some ether derivatives to their calix[4]arene analogs does not result in an increase in or acquisition of any antibacterial activity, at least at useful therapeutic levels. Nevertheless, the calixarene species **14** integrating two 2,2′-bithiazole-5,5′-dicarboxylate subunits exhibits activity against the *S. aureus* ATCC 25923 strain. It is under current investigation to determine the role of its structure (acetate and carboxylated 2,2′-bithiazole groups) in relation to its selective activity, and to develop more potent derivatives.

## Experimental Part

*General. Chemistry:* All commercially available products were used without further purification unless otherwise specified. TLC: silica gel plates ($SiO_2$, ref. 1.05554; $Al_2O_3$, ref. 1.05581, *Merck*). M.p.: *Electrothermal 9200* cap. apparatus; uncorrected. UV/VIS Spectra : *SAFAS UV mc²* apparatus, $\lambda_{max}$ in nm, $\varepsilon$ in $mol^{-1}$ $dm^3$ $cm^{-1}$. IR Spectra: *Bruker Vector 22* apparatus (KBr); $\tilde{\nu}$ in $cm^{-1}$. $^1$H- and $^{13}$C-NMR spectra: *Bruker DRX 400*; $\delta$ in ppm, *J* in Hz. ES-MS: *Micromass Platform II* apparatus, at the Service Commun de Spectrométrie de Masse Organique, Nancy; in *m*/*z*. Elemental analyses: Service de Microanalyse, Nancy. Dialysis were performed on *Float-A-Lyser*, cellulose acetate, molecular weight cut off (MWCO) 100 D.

For each calixarene derivative, *A* denotes the unsubstituted phenol ring, *B* the substituted one; C-atom numbering in heterocycles was defined as shown in *Scheme 1*.

*Biology.* The drugs were prepared in sterile dist. $H_2O$ and inoculums in *Mueller–Hinton* medium. Bacteria were grown in *Mueller–Hinton* broth (*Difco*, 275730), or *Mueller–Hinton* agar (*Difco*, 225250); purity of isolates was checked at the time of every test by examination of colony morphology and *Gram* staining. For *MIC* determination experiments, bacteria were inoculated in 96-well U shape microtiter plates to yield a final inoculum of $1 \times 10^5$ colony-forming units (CFU)/ml. Then, various concentrations of the drugs were added. The cultures were grown for 18–24 h at 35°. The resulting bacterial growth was measured with an ELISA plate reader (*Multiskan EX*, *Thermo Electron Corporation*, France) at a wavelength of 540 nm. Each *MIC* value was determined three times, involving three blanks (medium, medium + drug, medium + bacteria).

*Calixarene Esters: General Procedure for Compounds* **7** *and* **9**. A suspension of tetraethyl ester **2** (1 equiv.) and $K_2CO_3$ (1 equiv.) in dry MeCN was refluxed under Ar during 30 min. The halogenomethyl derivative (2 equiv.) was then added, and reflux was continued during *ca.* 6 h (TLC monitoring). After cooling to r.t., the solvent was evaporated to dryness, and the resulting solid material was dissolved in $CH_2Cl_2$. The insoluble inorg. materials were filtered off, and the org. phase was concentrated and then chromatographed to give the substituted ester.

*Tetraethyl 25,27-Dihydroxy-26,28-bis[(6′-methyl-2,2′-bipyridin-6-yl)methoxy]calix[4]arene-5,11,17,23-tetraacetate* (= *Tetraethyl 2,2′,2″,2‴-{25,27-Dihydroxy-26,28-bis[(6′-methyl-2,2′-bipyridin-6-yl)methoxy]pentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-5,11,17,23-tetrayl}tetraacetate*; **7**). From **2** (0.1 g, 0.13 mmol), $K_2CO_3$ (0.018 g, 0.13 mmol), MeCN (10 ml), and *6-(bromomethyl)-6′-methyl-2,2′-bipyridine* (**3**; 0.068 g, 0.26 mmol). TLC Monitoring: $SiO_2$; $CH_2Cl_2$/MeOH 98:2. Chromatography: *Chromatotron*, $Al_2O_3$, 1-mm thickness, $CH_2Cl_2$/hexane 4:1: **7** (0.09 g, 0.074 mmol; 57%). White powder. M.p. 90–91°. IR (KBr): 1733.1 (C=O). UV/VIS ($CH_2Cl_2$): 286 (49226). $^1$H-NMR (400 MHz, $CDCl_3$): 1.21 (*t*, *J* = 7.3, 2 *Me*$CH_2$O of *B*); 1.27 (*t*, *J* = 7.3, 2 *Me*$CH_2$O of *A*); 2.65 (*s*, 2 *Me*–bpy); 3.33 (*s*, 2 C$H_2$COOEt of *B*); 3.43, 4.42 (*AB*, $J_{AB}$ = 13.1, 4 ArC$H_2$Ar); 3.51 (*s*, 2 C$H_2$COOEt of *A*); 4.07–4.20 (*m*, 4 MeC$H_2$O); 5.23 (*s*, 2 OC$H_2$–bpy); 6.88 (*s*, 4 arom. H of *B*); 7.04 (*s*, 4 arom. H of *A*); 7.13 (*d*, *J* = 7.6, 2 H–C(5′) of bpy); 7.57–7.66 (*m*, 2 H–C(4), 2 H–C(4′) of bpy); 8.04–8.11 (*m*, 2 H–C(5) of bpy, 2 OH); 8.20 (*d*, *J* = 7.8, 2 H–C(3′) of bpy); 8.38 (*d*, *J* = 7.3, 2 H–C(3) of bpy). $^{13}$C-NMR (100 MHz, $CDCl_3$): 14.5 (*Me*$CH_2$O of *B*); 14.7 (*Me*$CH_2$O of *A*); 25.0

(*Me*–bpy); 32.0 (ArCH$_2$Ar); 41.0 (*C*H$_2$COOEt of *A*); 41.3 (*C*H$_2$COOEt of *B*); 61.1, 61.2 (MeCH$_2$O of *A* and *B*); 79.2 (OCH$_2$–bpy); 118.7 (C(3′) of bpy); 120.5 (C(3) of bpy); 121.4 (C(5) of bpy); 123.7 (C(5′) of bpy); 124.8 (C$_p$ of *A*); 128.1 (C$_o$ of *A*); 129.9 (C$_m$ of *A*); 130.6 (C$_m$ of *B*); 131.3 (C$_p$ of *B*); 133.5 (C$_o$ of *B*); 137.5 (C(4′) of bpy); 138.6 (C(4) of bpy); 151.7 (C$_{ipso}$ of *B*); 153.0 (C$_{ipso}$ of *A*); 155.7 (C(2′) of bpy); 156.3 (C(2) of bpy); 156.6 (C(6) of bpy); 158.3 (C(6′) of bpy); 172.1 (*C*OOEt of *B*); 172.6 (*C*OOEt of *A*). ES-MS (pos.): 1133.94 ([*M* + H]$^+$). Anal. calc. for $C_{68}H_{68}N_4O_{12} \cdot CH_2Cl_2$ (1218.23): C 68.03, H 5.79, N 4.59; found: C 67.83, H 5.71, N 4.66.

*Tetraethyl 25,27-Dihydroxy-26,28-bis{[4,4′-bis(methoxycarbonyl)-6′-methyl-2,2′-bipyridin-6-yl]methoxy}calix[4]arene-5,11,17,23-tetraacetate* (= *Tetramethyl 6,6′-{[5,11,17,23-Tetrakis(2-ethoxy-2-oxoethyl)-26,28-dihydroxypentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diyl]bis(oxymethanediyl)}bis(6′-methyl-2,2′-bipyridine-4,4′-dicarboxylate)*; **9**). From **2** (0.1 g, 0.13 mmol), $K_2CO_3$ (0.018 g, 0.13 mmol), MeCN (10 ml), *dimethyl 6-(bromomethyl)-6′-methyl-2,2′-bipyridine-4,4′-dicarboxylate* (**5**; 0.098 g, 0.26 mmol). TLC Monitoring: $Al_2O_3$, $CH_2Cl_2$. After filtration of insoluble inorg. materials, addition to the $CH_2Cl_2$ soln. of an excess MeOH resulted in the formation of a gel that was filtered and dried prior chromatography. Chromatography: column, $Al_2O_3$; $CH_2Cl_2$: **9** (0.102 g, 0.075 mmol, 57%). White powder. M.p. 108–109°. IR (KBr): 1733.2 (C=O). UV/VIS ($CH_2Cl_2$): 286 (61139). $^1$H-NMR (400 MHz, $CDCl_3$): 1.22 (*t*, *J* = 7.3, 2 *Me*CH$_2$O of *A* or *B*); 1.28 (*t*, *J* = 7.3, 2 *Me*CH$_2$O of *B* or *A*); 2.76 (*s*, 2 *Me*–bpy); 3.38 (*s*, 2 C*H*$_2$COOEt of *B*); 3.45 (*s*, 2 C(4′)–COO*Me*); 3.47, 4.41 (*AB*, $J_{AB}$ = 13.1, 4 ArC*H*$_2$Ar); 3.51 (*s*, 2 C*H*$_2$COOEt of *A*); 3.98 (*s*, 2 C(4)–COO*Me*); 4.09–4.21 (*m*, 4 MeC*H*$_2$O); 5.42 (*s*, 2 OC*H*$_2$–bpy); 6.94 (*s*, 4 arom. H of *B*); 7.05 (*s*, 4 arom. H of *A*); 7.76 (*s*, 2 H–C(5′) of bpy); 8.32 (*s*, 2 OH); 8.74 (*s*, 2 H–C(3′) of bpy); 8.78 (*s*, 2 H–C(3) of bpy); 9.10 (*s*, 2 H–C(5) of bpy). $^{13}$C-NMR (100 MHz, $CDCl_3$): 14.6, 14.7 (*Me*CH$_2$O of *A* and *B*); 25.0 (*Me*–bpy); 32.1 (Ar*C*H$_2$Ar); 41.0 (*C*H$_2$COOEt of *A*); 41.3 (*C*H$_2$COOEt of *B*); 52.6 (C(4)–COO*Me*); 53.1 (C(4′)–COO*Me*); 61.1, 61.2 (Me*C*H$_2$O of *A* and *B*); 78.8 (O*C*H$_2$–bpy); 117.9 (C(3′) of bpy); 120.0 (C(3) of bpy); 121.8 (C(5) of bpy); 123.3 (C(5′) of bpy); 124.8 (C$_p$ of *A*); 127.9 (C$_o$ of *A*); 129.8 (C$_m$ of *A*); 130.7 (C$_m$ of *B*); 131.5 (C$_p$ of *B*); 133.6 (C$_o$ of *B*); 139.1, 139.7 (C(4), C(4′) of bpy); 151.7 (C$_{ipso}$ of *B*); 153.1 (C$_{ipso}$ of *A*); 156.1 (C(2′) of bpy); 156.2 (C(2) of bpy); 158.7 (C(6) of bpy); 159.8 (C(6′) of bpy); 165.4 (C(4′)–COOMe); 166.4 (C(4)–COOMe); 172.1 (*C*OOEt of *B*); 172.6 (*C*OOEt of *A*). ES-MS (pos.): 1364.75 ([*M* + H]$^+$). Anal. calc. for $C_{76}H_{76}N_4O_{20}$ (1365.44): C 66.85, H 5.61, N 4.10; found: C 66.95, H 5.60, N 4.12.

*Calixarene Salts* via *Acids: General Procedure for Compounds* **11**–**15**. The ester (1 equiv.) was suspended in a 1:1 mixture EtOH/$H_2O$. Solid NaOH (40 equiv.) was added to the mixture, which was maintained at reflux (TLC monitoring). The soln. was cooled to r.t., and EtOH was evaporated under vacuum; the resulting aq. soln. was acidified with 1M HCl, affording a precipitate that was filtered (with or without centrifugation) and dried ($P_2O_5$) to give the corresponding acid. The latter was suspended in dist. $H_2O$, and 0.1M aq. NaOH was carefully added until solubilization was complete (pH-meter monitoring). The resulting soln. was filtered and then lyophilized. The resulting solid was dissolved in dist. $H_2O$, dialyzed, and finaly lyophilized to give the corresponding sodium salt.

*Tetrasodium 25,27-Dihydroxy-26,28-bis[(6′-methyl-2,2′-bipyridin-6-yl)methoxy]calix[4]arene-5,11,17,23-tetraacetate* (= *Tetrasodium 2,2′,2″,2‴-{25,27-Dihydroxy-26,28-bis[(6′-methyl-2,2′-bipyridin-6-yl)methoxy]pentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-5,11,17,23-tetrayl}tetraacetate*; **11**). From **7** (0.24 g, 0.197 mmol), EtOH/$H_2O$ (30 ml), and NaOH (0.34 g, 8.61 mmol), reflux 7 h. TLC Monitoring ($Al_2O_3$; $CH_2Cl_2$): tetra acid (0.21 g, 92%), then: $H_2O$ (30 ml), 0.1M NaOH; final pH 8.25: **11** (0.206 g, 78%). Woolly white powder. Tetra acid: White solid. $^1$H-NMR (100 MHz, ($D_6$)DMSO): 2.55 (*s*, 2 *Me*–bpy); 3.27 (*s*, 2 C*H*$_2$COOH of *A* or *B*); 3.38 (*s*, 2 C*H*$_2$COOH of *A* or *B*); 3.44, 3.32 (*AB*, $J_{AB}$ = 13.1, 4 ArC*H*$_2$Ar); 5.16 (*s*, 2 OC*H*$_2$–bpy); 6.93 (*s*, 4 arom. H of *A* or *B*); 7.07 (*s*, 4 arom. H of *A* or *B*); 7.24 (*d*, *J* = 7.3, 2 arom. H of bpy); 7.60–7.72 (*m*, 4 arom. H of bpy); 7.81 (*d*, *J* = 7.5, 2 arom. H of bpy); 8.19 (*d*, *J* = 8.0, 2 arom. H of bpy); 8.29–8.35 (*m*, 6 arom. H of bpy, 2 OH). Salt **11**: see [38].

*Octasodium 25,27-Bis[(4,4′-dicarboxy-6′-methyl-2,2′-bipyridin-6-yl)methoxy]-26,28-dihydroxycalix[4]arene-5,11,17,23-tetraacetate* (= *Octasodium 6,6′-{[5,11,17,23-Tetrakis(carboxylatomethyl)-26,28-dihydroxypentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diyl]bis(oxymethanediyl)}bis(6′-methyl-2,2′-bipyridine-4,4′-dicarboxylate)*; **13**). From **9** (0.29 g, 0.21 mmol), EtOH/$H_2O$ (20 ml), and NaOH (0.34 g, 8.61 mmol), reflux 24 h. TLC Monitoring ($Al_2O_3$,

$CH_2Cl_2$), centrifugation: octa-acid (0.22 g, 94%); then: $H_2O$ (40 ml), 0.1M NaOH, initial pH 2.30, final pH 7.92: **13** (0.26 g, 53%). Woolly light yellow powder. Octa-acid: Light yellow powder. $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.66 (*s*, 2 *Me*–bpy); 3.10–3.66 (*m*, 4 H of Ar$CH_2$Ar, 4 $CH_2$COOH); 4.28 (*d*, *J* = 12.8, 4 H of Ar$CH_2$Ar); 5.38 (*s*, 2 O$CH_2$–bpy); 6.96 (*s*, 4 arom. H of *A* or *B*); 7.07 (*s*, 4 arom. H of *A* or *B*); 7.71, 8.50, 8.53, 8.65, 9.07 (5 *s*, 8 arom. H of bpy, 2 OH). Salt **13**: see [38].

*Tetrasodium 25,27-Dihydroxy-26,28-bis[(4′-methyl-2,2′-bithiazol-4-yl)methoxy]calix[4]arene-5,11,17,23-tetraacetate* (= *Tetrasodium 2,2′,2″,2‴-{25,27-Dihydroxy-26,28-bis[(4′-methyl-2,2′-bithiazol-4-yl)methoxy]pentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-5,11,17,23-tetrayl}tetraacetate*; **12**). In addition to [30]: flame photometry: calc. for $C_{52}H_{40}N_4Na_4O_{12}S_4 \cdot 6\ H_2O \cdot NaCl$ (1299.60): Na 8.84; found: Na 8.95. ES-MS (neg.): 1109.58 ([*M* – Na]$^-$), 1087.59 ([*M* – 2 Na + H]$^-$), 1065.61 ([*M* – 3 Na + 2 H]$^-$), 1043.62 ([*M* – 4 Na + 3 H]$^-$), 543.24 ([*M* – 2 Na]$^{2-}$), 532.16 ([*M* – 3 Na + H]$^{2-}$), 521.19 ([*M* – 4 Na + 2 H]$^{2-}$).

*Octasodium 25,27-Bis[(5,5′-dicarboxy-4′-methyl-2,2′-bithiazol-4-yl)methoxy]-26,28-dihydroxycalix[4]arene-5,11,17,23-tetraacetate* (= *Octasodium 4,4′-{[5,11,17,23-Tetrakis(carboxylatomethyl)-26,28-dihydroxypentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diyl]bis(oxymethanediyl)}bis(4′-methyl-2,2′-bithiazole-5,5′-dicarboxylate)*; **14**). In addition to [30]: flame photometry: calc. for $C_{56}H_{36}N_4Na_8O_{20}S_4 \cdot 5\ NaCl \cdot 7\ H_2O$ (1815.40): Na 16.45; found: Na 16.08. ES-MS (neg.): 685.88 ([*M* – Na + H]$^{2-}$), 674.92 ([*M* – 2 Na]$^{2-}$), 663.90 ([*M* – 3 Na + H]$^{2-}$), 652.93 ([*M* – 4 Na + 2 H]$^{2-}$), 641.97 ([*M* – 5 Na + 3 H]$^{2-}$), 631.01 ([*M* – 6 Na + 4 H]$^{2-}$), 619.92 ([*M* – 7 Na + 5 H]$^{2-}$), 609.02 ([*M* – 8 Na + 6 H]$^{2-}$), 598.00 ([*M* – 5 Na + 3 H – 2 (COO)]$^{2-}$), 586.97 ([*M* – 6 Na + 4 H – 2 (COO)]$^{2-}$), 575.95 ([*M* – 7 Na + 5 H – 2 (COO)]$^{2-}$), 564.99 ([*M* – 8 Na + 6 H – 2 (COO)]$^{2-}$), 554.02 ([*M* – 5 Na + 3 H – 4 (COO)]$^{2-}$), 543.06 ([*M* – 6 Na + H – 4 (COO)]$^{2-}$), 532.04 ([*M* – 7 Na + 5 H – 4 (COO)]$^{2-}$), 521.07 ([*M* – 8 Na + 6 H – 4 (COO)]$^{2-}$).

*Tetrasodium 25,27,26,28-Tetrahydroxycalix[4]arene-5,11,17,23-tetraacetate* (= *Tetrasodium 2,2′,2″,2‴-[25,26,27,28-Tetrahydroxypentacyclo[19.3.1.1$^{3,7}$.1$^{9,13}$.1$^{15,19}$]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-5,11,17,23-tetrayl]tetraacetate*; **15**). In addition to [30]: flame photometry: calc. for $C_{36}H_{28}Na_4O_{12} \cdot 1.5\ NaCl \cdot 5.5\ H_2O$ (931.21): Na 13.58; found: Na 14.03.

*Monomer Esters: General Procedure for Compounds* **18**–**21**. A suspension of *methyl 2-(4-hydroxyphenyl)acetate* (**16**; 1 equiv.) and $K_2CO_3$ (1 equiv.) in dry MeCN was refluxed under Ar during 30 min. The halogenoalkyl derivative was then added, and reflux was continued during *ca.* 4 h (TLC monitoring). After cooling to r.t., the solvent was evaporated to dryness, and the resulting solid material was dissolved in $CH_2Cl_2$. The inorg. materials were filtered off, and the org. phase was chromatographed to give the desired ester.

*Methyl 2-{4-[(6′-Methyl-2,2′-bipyridin-6-yl)methoxy]phenyl}acetate* (**18**). From **16** (0.1 g, 0.60 mmol), $K_2CO_3$ (0.08 g, 0.60 mmol), MeCN (30 ml), **3** (0.16 g, 0.60 mmol), reflux 4 h (TLC monitoring: $Al_2O_3$; $CH_2Cl_2$). Chromatography: $Al_2O_3$; $CH_2Cl_2$: **18** (0.16 g, 0.46 mmol, 77%). White powder. M.p. 90–91°. IR (KBr): 1731.2 (C=O). UV/VIS ($CH_2Cl_2$): 288 (16805). $^1$H-NMR (400 MHz, $CDCl_3$): 2.65 (*s*, *Me*–bpy); 3.57 (*s*, $CH_2$COOMe); 3.68 (*s*, COO*Me*); 5.27 (*s*, O$CH_2$–bpy); 6.98 (*d*, *J* = 8.6, 2 arom. H); 7.15–7.24 (*m*, 3 arom. H of $C_6H_4$, bpy); 7.51 (*d*, *J* = 7.5, 1 H of bpy); 7.72 (*t*, *J* = 7.7, 1 H of bpy); 7.82 (*t*, *J* = 7.8, 1 H of bpy); 8.20 (*d*, *J* = 7.8, 1 H of bpy); 8.34 (*d*, *J* = 7.8, 1 H of bpy). $^{13}$C-NMR (100 MHz, $CDCl_3$): 25.1 (*Me*–bpy); 40.7 ($CH_2$COOMe); 52.4 (COO*Me*); 71.3 (O$CH_2$–bpy); 115.4 ($C_m$); 118.6, 120.4, 121.4, 123.7 (C(3,3′,5,5′) of bpy); 126.9 ($C_p$); 130.8 ($C_o$); 137.5, 138.0 (C(4,4′) of bpy); 157.2 ($C_{ipso}$); 155.8, 156.3, 158.1, 158.3 (C(6,6′,2,2′) of bpy); 172.7 (C=O). ES-MS (pos.): 347.70 ([*M* + H]$^+$), 289.40 ([*M* – COOMe + H]$^+$). Anal. calc. for $C_{21}H_{20}N_2O_3 \cdot 0.1\ H_2O$ (350.20): C 72.02, H 5.81, N 8.00; found: C 71.89, H 5.81, N 7.74.

*Methyl 2-{4-[(4′-Methyl-2,2′-bithiazol-4-yl)methoxy]phenyl}acetate* (**19**). From **16** (0.1 g, 0.60 mmol), $K_2CO_3$ (0.08 g, 0.60 mmol), MeCN (30 ml), 4-(bromomethyl)-4′-methyl-2,2′-bithiazole (**4**; 0.16 g, 0.58 mmol), reflux 7 h (TLC monitoring: $Al_2O_3$; $CH_2Cl_2$). Chromatography: $Al_2O_3$, $CH_2Cl_2$: **19** (0.10 g, 0.27 mmol, 46%). White powder. M.p. 101–102°. IR (KBr): 1742.1 (C=O). UV/VIS ($CH_2Cl_2$): 330 (16950). $^1$H-NMR (400 MHz, $CDCl_3$): 2.53 (*s*, *Me*–btz); 3.58 (*s*, $CH_2$COOMe); 3.69 (*s*, COO*Me*); 5.24 (*s*, O$CH_2$–btz); 6.96 (*d*, *J* = 8.3, 2 arom. H); 7.00 (*s*, 1 H of btz); 7.21 (*d*, *J* = 8.1, 2 arom. H); 7.40 (*s*, 1 H of btz). $^{13}$C-NMR (100 MHz, $CDCl_3$): 17.5 (*Me*–btz); 40.7 ($CH_2$COOMe); 52.4 ($CH_2$COO*Me*); 66.7 (O$CH_2$–btz); 115.3 ($C_m$); 116.3, 118.1 (C(5,5′) of btz); 127.1 ($C_p$); 130.8 ($C_o$); 157.9 ($C_{ipso}$); 154.2, 154.7,

160.8, 162.2 (C(2,2′,4,4′) of btz); 172.6 (C=O). ES-MS (pos.): 360.00 ($[M+H]^+$). Anal. calc. for $C_{17}H_{16}N_2O_3S_2 \cdot 0.5\ H_2O$ (369.45): C 55.26, H 4.64, N 7.58, S 17.35; found: C 55.86, H 4.40, N 7.68, S 17.47.

*Dimethyl 6-{[4-(2-Methoxy-2-oxoethyl)phenoxy]methyl}-6′-methyl-2,2′-bipyridine-4,4′-dicarboxylate* (**20**). From **16** (0.24 g, 1.45 mmol), $K_2CO_3$ (0.20 g, 1.45 mmol), MeCN (50 ml), **5** (0.55 g, 1.45 mmol), reflux 7 h (TLC monitoring: $Al_2O_3$; $CH_2Cl_2$/hexane 90:10). Chromatography: $Al_2O_3$; $CH_2Cl_2$/hexane 9:1: **20** (0.67 g, 1.41 mmol, 97%). White powder. M.p. 160–161°. IR (KBr): 1736.8 (C=O). UV/VIS ($CH_2Cl_2$): 310 (17074). $^1$H-NMR (400 MHz, $CDCl_3$): 2.79 (*s*, *Me*–bpy), 3.60 (*s*, C$H_2$COOMe); 3.72 (*s*, CH$_2$COO*Me*); 4.03 (*s*, COO*Me*–bpy); 4.03 (*s*, COO*Me*–bpy); 5.37 (*s*, OC$H_2$–bpy); 7.04 (*d*, *J* = 8.8, 2 arom. H); 7.25 (*d*, *J* = 8.8, 2 arom. H); 7.82 (*s*, 1 H of bpy); 8.15 (*s*, 1 H of bpy); 8.79 (*s*, 1 H of bpy); 8.94 (*s*, 1 H of bpy). $^{13}$C-NMR (100 MHz, $CDCl_3$): 25.0 (*Me*–bpy); 40.7 (*C*H$_2$COOMe); 52.4 (CH$_2$COO*Me*); 53.1 (COO*Me*–bpy); 53.1 (COO*Me*–bpy); 71.0 (OCH$_2$–bpy); 115.4 ($C_m$ or $C_o$); 118.1, 120.0, 121.0, 123.3 (C(3,3′,5,5′) of bpy); 127.2 ($C_p$); 130.8 ($C_m$ or $C_o$); 139.1, 139.8 (C(4,4′) of bpy); 157.9 ($C_{ipso}$); 156.1, 156.6, 158.7, 159.8 (C(2,2′,6,6′) of bpy); 166.1, 166.4 (C=O of bpy); 172.6 (C=O). ES-MS (pos.): 463.00 ($[M+H]^+$), 405.00 ($[M-COOMe+H]^+$), 300.00 ($[bpy(COOMe)_2+H]^+$). Anal. calc. for $C_{25}H_{24}N_2O_7 \cdot 0.5\ H_2O$ (473.47): C 63.42, H 5.32, N 5.91; found: C 63.49, H 5.08, N 5.89.

*Diethyl 4-{[4-(2-Methoxy-2-oxoethyl)phenoxy]methyl}-4′-methyl-2,2′-bithiazole-5,5′-dicarboxylate* (**21**). From **16** (0.20 g, 1.18 mmol), $K_2CO_3$ (0.15 g, 1.30 mmol), MeCN (30 ml), *diethyl 4-(bromomethyl)-4′-methyl-2,2′-bithiazole-5,5′-dicarboxylate* (**6**; 0.55 g, 1.30 mmol), reflux 4 h (TLC monitoring; $Al_2O_3$, $CH_2Cl_2$). Chromatography: $Al_2O_3$, $CH_2Cl_2$/hexane 3:1: **21** (0.43 g, 0.85 mmol, 72%). Yellow powder. M.p. 113–114°. IR (KBr): 1719.8 (C=O). UV/VIS ($CH_2Cl_2$): 350 (26193). $^1$H-NMR (400 MHz, $CDCl_3$): 1.35 (*t*, *J* = 7.0, *Me*CH$_2$O); 1.38 (*t*, *J* = 7.0, *Me*CH$_2$O); 2.78 (*s*, *Me*–btz); 3.57 (*s*, C$H_2$COOMe); 3.68 (*s*, COO*Me*), 4.30–4.40 (*m*, 2 MeC$H_2$O); 5.49 (*s*, OC$H_2$–btz); 7.01 (*d*, *J* = 8.6, 2 arom. H); 7.20 (*d*, *J* = 8.6, 2 arom. H). $^{13}$C-NMR (100 MHz, $CDCl_3$): 14.5, 14.6 (*Me*CH$_2$O); 17.9 (*Me*–btz); 40.7 (*C*H$_2$COOMe); 52.4 (COO*Me*); 62.0, 62.5 (COO*C*H$_2$Me); 64.4 (OCH$_2$–btz); 115.6 ($C_m$ or $C_o$); 125.4, 127.1, 128.4 (C(5,5′), $C_p$); 130.7 ($C_m$ or $C_o$); 158.2, 158.8, 161.3, 161.6, 161.8 (C(4,4′,2,2′), $C_{ipso}$); 162.2, 163.4 (*C*OOEt); 172.7 (*C*OOMe). ES-MS (pos.): 505.12 ($[M+H]^+$); 527.14 ($[M+Na]^+$). Anal. calc. for $C_{23}H_{24}N_2O_7S_2$ (504.58): C 54.75, H 4.79, N 5.55, S 12.70; found: C 54.76, H 4.67, N 5.58, S 12.80.

*Monomer Salts* via *Acids: General Procedure for Compounds* **26**–**30**. The ester (1 equiv.) was suspended in a mixture of EtOH and $H_2O$. A soln. of NaOH (10 equiv.) in $H_2O$ was added, and the resulting mixture was refluxed during 7 h. The soln. was cooled to r.t., and EtOH was evaporated under vacuum; the resulting aq. soln. was acidified with 1M HCl, affording the corresponding acid as a precipitate, which was filtered, then washed with $H_2O$, and dried. The acid derivative was then suspended in dist. $H_2O$, and 0.1M aq. NaOH was carefully added until solubilization was complete (pH-meter monitoring). The resulting soln. was filtered and then lyophilized to give the desired sodium salt.

*Sodium 2-{4-[(6′-Methyl-2,2′-bipyridin-6-yl)methoxy]phenyl}acetate* (**26**). From **18** (0.30 g, 0.86 mmol), EtOH/$H_2O$ 1:1 (30 ml), NaOH (0.34 g, 8.61 mmol) in $H_2O$ (10 ml): **22** (0.20 g, 0.60 mmol, 70%); then, **22** (0.17 g, 0.51 mmol), $H_2O$ (30 ml), 0.1M NaOH, final pH 7.70. **26** (0.19 g, 0.49 mmol, 96% *vs.* acid, 67% *vs.* **18**).

*Data of* **22**. White powder. IR (KBr): 1702.9 (C=O). $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.58 (*s*, *Me*–bpy); 3.49 (*s*, C$H_2$COOH); 5.26 (*s*, OC$H_2$–bpy); 7.01 (*d*, *J* = 8.6, 2 arom. H); 7.19 (*d*, *J* = 8.6, 2 arom. H); 7.33 (*d*, *J* = 7.6, 1 H of bpy); 7.55 (*d*, *J* = 7.4, 1 H of bpy); 7.84 (*t*, *J* = 7.8, 1 H of bpy); 7.97 (*t*, *J* = 7.7, 1 H of bpy); 8.20 (*d*, *J* = 7.8, 1 H of bpy); 8.32 (*d*, *J* = 7.8, 1 H of bpy). Anal. calc. for $C_{20}H_{18}N_2O_3$ (334.37): C 71.84, H 5.42, N 8.37; found: C 71.60, H 5.40, N 8.35.

*Data of* **26**. Woolly white powder. M.p. 200–201°. IR (KBr): 1583.1, 1571.9 (C=N and C=O). UV/VIS ($H_2O$): 286 (14346). $^1$H-NMR (400 MHz, $D_2O$): 2.62 (*s*, *Me*–bpy); 3.49 (*s*, C$H_2$COOH); 5.33 (*s*, OC$H_2$–bpy); 7.07 (*d*, *J* = 7.8, 2 arom. H); 7.26 (*d*, *J* = 8.1, 2 arom. H); 7.41 (*d*, *J* = 8.1, 1 H of bpy); 7.63 (*d*, *J* = 7.8, 1 H of bpy); 7.79 (*d*, *J* = 7.8, 1 H of bpy); 7.85–7.95 (*m*, 2 H of bpy); 8.00 (*t*, *J* = 7.6, 1 H of bpy). $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.57 (*s*, *Me*–bpy); 3.14 (*s*, C$H_2$COOH); 5.22 (*s*, OC$H_2$–bpy); 6.90 (*d*, *J* = 8.1, 2 arom. H); 7.15 (*d*, *J* = 8.1, 2 arom. H); 7.32 (*d*, *J* = 7.5, 1 H of bpy); 7.54 (*d*, *J* = 7.5, 1 H of bpy); 7.84 (*t*, *J* = 7.7, 1 H of bpy); 7.96 (*t*, *J* = 7.7, 1 H of bpy); 8.20 (*d*, *J* = 7.8, 1 H of bpy); 8.31 (*d*, *J* = 7.8, 1 H of bpy). $^{13}$C-NMR (100 MHz, ($D_6$)DMSO): 24.6 (*Me*–bpy); 45.6 (*C*H$_2$COONa); 70.7 (OC$H_2$–bpy); 114.3 ($C_m$); 118.0, 119.7, 122.0, 124.0 (C(3,3′,5,5′) of bpy); 130.5 ($C_o$); 133.1 ($C_p$); 137.8, 138.4 (C(4,4′) bpy); 156.1 ($C_{ipso}$); 154.7, 155.3, 157.2, 157.9 (C(6,6′,2,2′) of bpy); 175.1 (COONa). ES-MS (pos.): 379.18 ($[M+$

Na]$^+$), 357.21 ([$M$ + H]$^+$), 335.30 ([$M$ – Na + 2 H]$^+$). ES-MS (neg.): 333.33 ([$M$ – Na]$^-$), 289.39 ([$M$ – COONa]$^-$), 183.41 ([$CH_2$–Bpy–Me]$^-$). Anal. calc. for $C_{20}H_{17}N_2NaO_3 \cdot 0.5$ NaCl (385.56): C 62.30, H 4.44, N 7.26; found: C 62.24, H 4.81, N 7.19.

*Sodium 2-{4-[(4′-Methyl-2,2′-bithiazol-4-yl)methoxy]phenyl}acetate* (**27**). From **19** (0.29 g, 0.79 mmol), EtOH/$H_2O$ 0.5 :1 (30 ml), NaOH (0.33 g, 8.18 mmol) in $H_2O$ (10 ml). Acidification and centrifugation: acid **23** (0.28 g, 0.77 mmol, 97%); then $H_2O$ (40 ml), 0.1M NaOH, final pH 8.5: **27** (0.30 g, 0.72 mmol, 94% *vs.* acid, 91% *vs.* **19**).

*Data of* **23**: Pale yellow powder. IR (KBr): 1720.5 (C=O). $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.44 (*s*, *Me*–btz); 3.50 (*s*, C$H_2$COOH); 5.20 (*s*, OC$H_2$–Btz); 7.01 (*d*, $J$ = 8.3, 2 arom. H); 7.20 (*d*, $J$ = 8.3, 2 arom. H); 7.51 (*s*, 1 H of btz); 7.89 (*s*, 1 H of btz); 12.24 (*s*, COOH). Anal. calc. for $C_{16}H_{14}N_2O_3S_2 \cdot$ 0.5 HCl (364.66): C 52.73, H 4.42, N: 7.68; found: C 52.74, H 4.33, N 7.66.

*Data of* **27**: Woolly white powder. M.p. 206 – 206°. IR (KBr): 1578 (br., C=N and C=O). UV/VIS ($H_2O$): 331 (14841). $^1$H-NMR (400 MHz, $D_2O$): 2.46 (*s*, *Me*–btz); 3.49 (*s*, C$H_2$COONa); 5.24 (*s*, OC$H_2$–Btz); 7.05 (*d*, $J$ = 8.1, 2 arom. H); 7.26 (*d*, $J$ = 8.3, 2 arom. H); 7.32 (*s*, 1 H of btz); 7.72 (*s*, 1 H of btz). $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.44 (*s*, *Me* – btz); 3.17 (*s*, C$H_2$COOH); 5.16 (*s*, OC$H_2$–Btz); 6.90 (*d*, $J$ = 7.5, 2 arom. H); 7.16 (*d*, $J$ = 7.5, 2 arom. H); 7.50 (*s*, 1 H of btz); 7.86 (*s*, 1 H of btz). $^{13}$C-NMR (100 MHz, ($D_6$)DMSO): 17.0 (*Me*–btz); 45.4 (*C*$H_2$COONa); 65.5 (OC$H_2$–btz); 114.3 ($C_m$); 117.6, 120.5 (C(5,5′) of btz); 130.4 ($C_o$); 133.0 ($C_p$); 156.0 ($C_{ipso}$); 153.8, 154.1, 159.9, 161.2 (C(2,2′,4,4′) of btz); 175.4 (COONa). ES-MS (pos.): 407.13 ([$M$ + K]$^+$), 391.12 ([$M$ + Na]$^+$), 369.15 ([$M$ + H]$^+$), 325.21 ([$M$ – COONa + Na + H]$^+$). ES-MS (neg.): 345.21 ([$M$ – Na]$^-$), 301.27 ([$M$ – COONa]$^-$), 195.29 ([$CH_2$–Btz–Me]$^-$). Anal. calc. for $C_{16}H_{13}N_2NaO_3S_2 \cdot 0.5$ NaCl $\cdot$ $H_2O$ (415.62): C 46.23, H 3.63, N 6.74, S 15.43; found: C 46.59, H 3.47, N 6.76, S 15.27.

*Trisodium 6-{[4-(Carboxylatomethyl)phenoxy]methyl}-6′-methyl-2,2′-bipyridine-4,4′-dicarboxylate* (**28**). From **20** (0.52 g, 1.10 mmol), EtOH/$H_2O$ 0.5 :1 (30 ml), NaOH (0.44 g, 11.2 mmol) in $H_2O$ (10 ml): acid **24** (0.46 g, 1.09 mmol, 99%); then $H_2O$ (30 ml), 0.1M NaOH, final pH 7.30: **28** (0.53 g, 0.86 mmol, 78%).

*Data of* **24**: White powder. $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.69 (*s*, *Me*–bpy), 3.50 (*s*, C$H_2$COOMe); 5.38 (*s*, OC$H_2$–bpy); 7.06 (*d*, $J$ = 8.1, 2 arom. H); 7.20 (*d*, $J$ = 7.8, 2 arom. H); 7.79 (*s*, 1 H of bpy); 7.98 (*s*, 1 H of bpy); 8.67 (*s*, 1 H of bpy); 8.77 (*s*, 1 H of bpy). Anal. calc. for $C_{22}H_{18}N_2O_7$ (422.39): C 62.55, H 4.29, N 6.63; found: C 62.71, H 4.31, N 6.73.

*Data of* **28**: Woolly white powder. M.p. > 290° (dec.). IR (KBr): 3406.3 (COONa), 1606.4, 1559.69 (br., C=N and C=O). UV/VIS ($H_2O$): 302 (12676). $^1$H-NMR (400 MHz, $D_2O$): 2.70 (*s*, *Me*–bpy), 3.50 (*s*, C$H_2$COOMe); 5.41 (*s*, OC$H_2$–bpy); 7.12 (*d*, $J$ = 8.1, 2 arom. H); 7.27 (*d*, $J$ = 8.1, 2 arom. H); 7.72 (*s*, 1 H of bpy); 7.99 (*s*, 1 H of bpy); 8.14 (*s*, 1 H of bpy); 8.27 (*s*, 1 H of bpy). $^{13}$C-NMR (100 MHz, $D_2O$): 23.5 (*Me*–bpy); 44.0 (*C*$H_2$COONa); 70.6 (OC$H_2$–bpy); 115.6 ($C_m$ or $C_o$); 119.4, 121.3, 121.7, 123.5 (C(3,3′,5,5′) bpy); 130.8 ($C_m$ or $C_o$); 131.1 ($C_p$); 146.8, 147.4 (C(4,4′) of bpy); 155.6, 156.6, 156.6, 159.9 (C(2,2′,6,6′) of bpy); 157.6 ($C_{ipso}$); 173.0, 173.5 (COONa of bpy); 181.7 ($CH_2$*C*OONa). ES-MS (pos.): 511.01 ([$M$ + Na]$^+$), 489.10 ([$M$ + H]$^+$), 445.10 ([$M$ – 2 Na + 3 H]$^+$). ES-MS (neg.): 465.16 ([$M$ – Na]$^-$), 443.19 ([$M$ – 2 Na + H]$^-$), 421.22 ([$M$ – 3 Na + 2 H]$^-$) or ([$M$ – COONa]$^-$), 399.00 ([$M$ – COONa – Na + H]$^-$), 377.27 ([$M$ – COONa – 2 Na + 2 H]$^-$), 354.29 ([$M$ – 2 COONa + H]$^-$), 332.31 ([$M$ – 2 COONa – Na + 2 H]$^-$), 288.37 ([$M$ – 3 COONa + 2 H]$^-$). Anal. calc. for $C_{22}H_{15}N_2Na_3O_7 \cdot$ NaCl $\cdot$ 4 $H_2O$ (618.81): C 42.70, H 3.74, N 4.52; found: C 42.67, H 3.43, N 4.54.

*Trisodium 4-{[4-(Carboxylatomethyl)phenoxy]methyl}-4′-methyl-2,2′-bithiazole-5,5′-dicarboxylate* (**29**). From **21** (0.38 g, 0.75 mmol), EtOH/$H_2O$ 3 :1 (20 ml), NaOH (0.30 g, 7.5 mmol) in $H_2O$ (10 ml): acid **25** (0.20 g, 0.46 mmol, 62%); then acid (0.19 g, 0.44 mmol), $H_2O$ (30 ml), 0.1M NaOH, final pH 7.90: **29** (0.20 g, 0.36 mmol, 82%).

*Data of* **25**: Pale yellow powder. IR (KBr): 1686.2 (COOH). $^1$H-NMR (400 MHz, ($D_6$)DMSO): 2.70 (*s*, *Me*–btz); 3.50 (*s*, C$H_2$COOH); 5.45 (*s*, OC$H_2$–btz); 6.99 (*d*, $J$ = 8.6, 2 arom. H); 7.19 (*d*, $J$ = 8.6, 2 arom. H). Anal. calc. for $C_{18}H_{14}N_2O_7S_2$ (434.45): C 49.76, H 3.24, N 6.44, S 14.76; found: C 49.73, H 3.28, N 6.42, S 14.80.

*Data of* **29**. Light yellow woolly powder. $^1$H-NMR (400 MHz, $D_2O$): 2.70 (*s*, *Me* – btz), 3.49 (*s*, C$H_2$COONa); 5.57 (*s*, OC$H_2$–btz); 7.08 (*d*, $J$ = 8.3, 2 arom. H); 7.25 (*d*, $J$ = 8.3, 2 arom. H). $^{13}$C-NMR

(100 MHz, $D_2O$): 16.2 (*Me*–btz); 43.9 (*C*$H_2$COONa); 64.1 (OC*H*$_2$–btz); 115.6 ($C_m$ or $C_o$); 130.7 ($C_m$ or $C_o$); 131.0, 133.7, 137.2 (C(5,5′), $C_p$); 153.4, 156.3, 156.6, 159.6, 161.1 (C(4,4′,2,2′) of btz, $C_{ipso}$); 167.2, 168.7 (COONa of btz); 181.7 (COONa). ES-MS (pos.): 523.00 ($[M+Na]^+$). ES-MS (neg.): 455.13 ($[M-2\ Na+H]^-$), 433.21 ($[M-3\ Na+2\ H]^-$) or ($[M-COONa]^-$), 411.22 ($[M-COONa-Na+H]^-$), 389.23 ($[M-COONa-2\ Na+2\ H]^-$), 367.30 ($[M-2\ COONa+H]^-$), 345.32 ($[M-2\ COONa-Na+2\ H]^-$). Anal. calc. for $C_{18}H_{11}N_2Na_3O_7S_2\cdot 3\ H_2O$ (554.39): C 38.99, H 3.09, N 5.05; found: C 39.32, H 2.86, N 5.03.

*Sodium 2-(4-Hydroxyphenyl)acetate* (**30**): *2-(4-Hydroxyphenyl)acetic acid* (**17**; 1 g, 6.57 mmol) was suspended in dist. $H_2O$ (40 ml), and 0.1M aq. NaOH was carefully added until solubilization was complete (final pH 8.0). The resulting soln. was filtered and then lyophilized to give **30** (1.14 g, 6.2 mmol, 94%). White woolly solid. $^1$H-NMR (400 MHz, $D_2O$): 3.46 (*s*, $CH_2$); 6.87 (*d*, $J=8.6$, 2 arom. H); 7.18 (*d*, $J=8.4$, 2 arom. H). $^1$H-NMR (400 MHz, ($D_6$)DMSO): 3.15 (*s*, $CH_2$); 6.63 (*d*, $J=8.3$, 2 arom. H); 7.00 (*d*, $J=8.3$, 2 arom. H). $^{13}$C-NMR (100 MHz, ($D_6$)DMSO): 45.2 (*C*$H_2$COONa); 115.0 ($C_o$ or $C_m$); 129.5 ($C_{ipso}$ or $C_p$); 130.2 ($C_o$ or $C_m$); 155.8 ($C_{ipso}$ or $C_p$); 176.5 (COONa). Anal. calc. for $C_8H_7NaO_3\cdot 0.25$ NaOH (184.09): C 52.18, H 3.97; found: C 52.46, H 3.98.

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## REFERENCES

[1] M. Leeb, *Nature* **2004**, *431*(7011), 892.
[2] A. de Fatima, S. A. Fernandes, A. A. Sabino, *Curr. Drug Discovery Technol.* **2009**, *6*, 151.
[3] R. V. Rodik, V. I. Boyko, V. I. Kalchenko, *Curr. Med. Chem.* **2009**, *16*, 1630.
[4] F. Perret, A. N. Lazar, A. W. Coleman, *Chem. Commun.* **2006**, 2425.
[5] F. Perret, A. W. Coleman, *Chem. Commun.* **2011**, *47*, 7303.
[6] J. W. Cornforth, P. D. Hart, G. A. Nicholls, R. J. W. Rees, J. A. Stock, *Br. J. Pharmacol.* **1955**, *10*, 73.
[7] P. D. Hart, J. A. Armstrong, E. Brodaty, *Infect. Immun.* **1996**, *64*, 1491.
[8] M. J. Colston, H. C. Hailes, E. Stavropoulos, A. C. Hervé, G. Hervé, K. J. Goodworth, A. M. Hill, P. Jenner, P. D. Hart, R. E. Tascon, *Infect. Immun.* **2007**, *72*, 6318.
[9] K. J. Goodworth, A.-C. Hervé, E. Stavropoulos, G. Hervé, I. Casades, A. M. Hill, G. G. Weingarten, R. E. Tascon, M. J. Colston, H. C. Hailes, *Tetrahedron* **2011**, *67*, 373.
[10] A. Casnati, M. Fabbi, N. Pelizzi, A. Pochini, F. Sansone, R. Ungaro, E. Di Modugno, G. Tarzia, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2699.
[11] M. Dudic, A. Colombo, F. Sansone, A. Casnati, G. Donofrio, R. Ungaro, *Tetrahedron* **2004**, *60*, 11613.
[12] F. Sansone, M. Dudic, G. Donofrio, C. Rivetti, L. Baldini, A. Casnati, S. Cellai, R. Ungaro, *J. Am. Chem. Soc.* **2006**, *128*, 14528.
[13] V. Bagnacani, F. Sansone, G. Donofrio, L. Baldini, A. Casnati, R. Ungaro, *Org. Lett.* **2008**, *10*, 3953.
[14] T. Yo, K. Fujiwara, M. Otsuka, JP 10203906, 1998.
[15] K. M. Hwang, Y. M. Qi, S. Y. Liu, W. Choy, J. Chen, WO Patent 9403164, 1994; K. M. Hwang, Y. M. Qi, S. Y. Liu, W. Choy, J. Chen, US Patent 5,441,983, 1995.
[16] S. J. Harris, WO Patent 9519974, 1995.
[17] S. J. Harris, WO Patent 0244121, 2002.
[18] D. Coveney, B. Costello, US Patent 2005113454, 2005.
[19] V. Kral, P. Cigler, J. Konvalinka, M. Kozisek, J. Prejdova, B. Gruener, J. Plesek, M. Lepsik, J. Pokorna, H.-G. Kraeusslich, J. Bodem, WO Patent 2005073240, 2005.
[20] R. P. M. Dings, X. Chen, D. M. E. I. Hellebrekers, L. I. van Eijk, Y. Zhang, T. R. Hoye, A. W. Griffioen, K. H. Mayo, *J. Natl. Cancer Inst.* **2006**, *98*, 932.
[21] A. E. Motornaya, L. M. Alimbarova, É. A. Shokova, V. V. Kovalev, *Pharm. Chem. J.* **2006**, *40*, 68.
[22] A. Ben Salem, J.-B. Regnouf-de-Vains, *Tetrahedron Lett.* **2001**, *42*, 7033; A. Ben Salem, J.-B. Regnouf-de-Vains, *Tetrahedron Lett.* **2003**, *44*, 6769; B. Korchowiec, A. Ben Salem, Y. Corvis, J.-B. Regnouf-de-Vains, J. Korchowiec, E. Rogalska, *J. Phys. Chem. B* **2007**, *111*, 13231.

[23] H. Massimba Dibama, I. Clarot, S. Fontanay, A. Ben Salem, M. Mourer, C. Finance, R. E. Duval, J.-B. Regnouf-de-Vains, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2679.
[24] M. Mourer, R. E. Duval, C. Finance, J.-B. Regnouf-de-Vains, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2960.
[25] M. Mourer, H. Massimba Dibama, S. Fontanay, M. Grare, R. E. Duval, C. Finance, J.-B. Regnouf-de-Vains, *Bioorg. Med. Chem.* **2009**, *17*, 5496.
[26] M. Grare, H. Massimba Dibama, S. Lafosse, A. Ribon, M. Mourer, C. Finance, J.-B. Regnouf-de-Vains, R. E. Duval, *Clin. Microbiol. Infect.* **2010**, *16*, 432.
[27] M. Grare, E. Dague, M. Mourer, J.-B. Regnouf-de-Vains, C. Finance, J.-F. L. Duval, R.-E. Duval, F. Gaboriaud, *Pathol. Biol.* **2007**, *55*, 465.
[28] M. Grare, S. Fontanay, H. Massimba Dibama, M. Mourer, J.-B. Regnouf-de-Vains, C. Finance, R. E. Duval, *Pathol. Biol.* **2010**, *58*, 46.
[29] M. Mourer, G. Laumond, A.-M. Aubertin, J.-B. Regnouf-de-Vains, unpublished results.
[30] M. Mourer, N. Psychogios, G. Laumond, A.-M. Aubertin, J.-B. Regnouf-de-Vains, *Bioorg. Med. Chem.* **2010**, *18*, 36.
[31] K. M. Hwang, Y. M. Qi, S. Y. Liu, T. C. Lee, W. Choy, J. Chen, WO 9403165 1994; K. M. Hwang, Y. M. Qi, S. Y. Liu, T. C. Lee, W. Choy, J. Chen, US Patent 5,409,959, 1995.
[32] G. Tuncel, C. Nergiz, *Lett. Appl. Microbiol.* **1993**, *17*, 300.
[33] T. Yasushi, A. Sugita, JP Patent, 2008100923 (*Chem. Abstr.* **2008**, *148*, 479114).
[34] J.-C. Rodrigez-Ubis, B. Alpha, D. Plancherel, J.-M. Lehn, *Helv. Chim. Acta* **1984**, *64*, 2264.
[35] N. Psychogios, J.-B. Regnouf-de-Vains, H. Stoekly-Evans, *Eur. J. Inorg. Chem.* **2004**, 2514.
[36] S. Pellet-Rostaing, J.-B. Regnouf-de-Vains, R. Lamartine, *Tetrahedron Lett.* **1996**, *37*, 5889.
[37] J.-M. Lehn, J.-B. Regnouf de Vains, *Tetrahedron Lett.* **1989**, *30*, 2209.
[38] M. Mourer, J.-B. Regnouf-de-Vains, *Tetrahedron Lett.* **2006**, *47*, 1895.
[39] C. D. Gutsche, K. C. Nam, *J. Am. Chem. Soc.* **1988**, *110*, 6153; S. K. Sharma, S. Kanamathareddy, C. D. Gutsche, *Synthesis* **1997**, 1268.
[40] C. Jaime, J. de Mendoza, P. Prados, P. M. Nieto, C. Sánchez, *J. Org. Chem.* **1991**, *56*, 3372; J. O. Magrans, J. de Mendoza, M. Pons, P. Prados, *J. Org. Chem.* **1997**, *62*, 4518.
[41] M. Grare, M. Mourer, J.-B. Regnouf-de-Vains, C. Finance, R.-E. Duval, *Pathol. Biol.* **2006**, *54*, 470.
[42] CA-SFM, 'Communiqué 2008', edn. 01.2008, http://www.sfm.asso.fr.; NCCLS, 'Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A6', 6th edn., 2003, National Committee for Clinical Laboratory Standards, Wayne, PA, USA.

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