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ORIGINAL ARTICLE

New macrocyclic compounds with naphthyridine units for molecular recognition studies of biotin and urea derivatives

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Abstract Two macrocyclic hosts containing benzenedicarboxamide or pyridinedicarboxamide moieties and two 1,8-naphthyridine units linked by a crown ether like chain, have been synthesized and fully characterized by multinuclear NMR spectroscopy. X-ray diffraction analysis is provided for one of the macrocycles including a DMSO guest molecule. Binding constant determination of both hosts with four ureido derivatives, amongst them (+)-biotin methyl ester, was achieved by means of ^{1}H NMR titrations.

Keywords Bisnaphthyridineamides · Benzenedicarboxamides · Pyridinedicarboxamides · Host–guest · Binding constants · MM calculations

Introduction

Macrocycles research has continually increased since Pedersen reported the synthesis of crown ethers in 1967. The interest in these compounds range from methodological aspects of their synthesis to a large number of applications in important areas such as catalysis, membrane transport, switches, sensors and biochemical systems, as demonstrated by the large number of contributions devoted to the subject. Molecular recognition studies are usually performed to understand features like complementarity in size, shape, and functional groups responsible for the macrocycles properties [1].

In the particular area of design of receptors for bioactive substances, ureas and biotin stand out for their significance. Biotin is a cofactor found in many enzymes that have diverse metabolic functions and is also used as a tag in different biological assays due to its capability of forming strong complexes via hydrogen bond (HB) with avidin ($K_b = 2.5 \times 10^{15}$ M^{-1}) or streptavidin ($K_b = 1.7 \times 10^{15}$ M^{-1}). The avidin–biotin binding is the strongest protein–ligand interaction reported and is a consequence of a combination of two factors: (i) a perfect fit of biotin through van der Waals interactions in a hydrophobic pocket formed by tryptophan and phenylalanine residues and (ii) the binding of biotin by the formation of 10 additional hydrogen bonds [2, 3].

Synthetic receptors that try to mimic this system tuning these two main non-covalent interactions have been reported. Wilcox and coworkers [4] described a Troger's base that binds biotin methyl ester exclusively by HB interactions. Goswami and Dey [5] reported two neutral isophthaloyl pyridine bisamide receptors with one and two methyl amido pyridine pendant arms, designed for tandem binding of biotin. We studied similar receptors for biotin methyl ester and urea derivatives fully soluble in chloroform, a solvent having comparable polarity to that of the interior cavity of an enzyme [6], and Ghosh and Sen [7] a benzothiazole-based receptor for recognition of biotin ester and urea.

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On the other hand, the introduction of the 1,8-naphthyridine moiety provides extra HB acceptor sites for complexation. Zimmerman and coworkers [8, 9] and Hamilton and Pant [10] were among the first groups to use 7-amido-1,8-naphthyridines to bind guanine derivatives. Our group [11–13], as well as Ghosh and Sen [14], have also contributed to this field using isophthaloyl or pyridine naphthyridyl bisamide systems to build receptors for methyl biotin ester and various ureas. Others have used the same motif to construct macrocycles [15–17], hydrogen bonded polymers [18, 19] or monosaccharide and disaccharide receptors [20, 21].

The aim of the present work has been to synthesize two new symmetrical macrocycles, **Ia** and **Ib**, with benzenedicarboxamide or pyridinedicarboxamide moieties and two 1,8-naphthyridine units linked by a chain similar to that found in crown ethers of 17 atoms with pendant nosyl groups (Fig. 1). These systems appear to be suitable for molecular recognition studies of ureas in low polarity solvents such as chloroform and dichloromethane, and they potentially offer recognition sites, HB and π-stacking and van der Waals interactions, for the ureido functionality and for the side chain of (+)-biotin methyl ester.

Bearing this in mind, the binding properties of the artificial receptors **Ia** and **Ib** have been studied with four guests: (+)-biotin methyl ester (**1**), 2-imidazolidinone (**2**), *N*,*N*'-trimethyleneurea (**3**), and barbital (**4**) (Fig. 2).

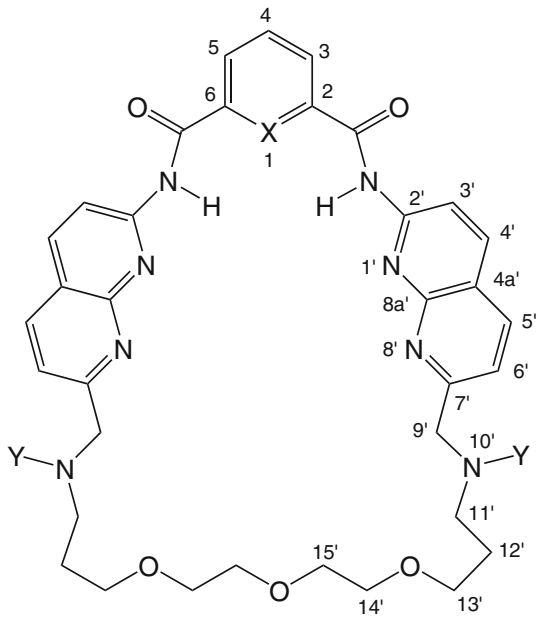


Fig. 1 Macrocyclic receptors **Ia** and **Ib**

Experimental section

Materials

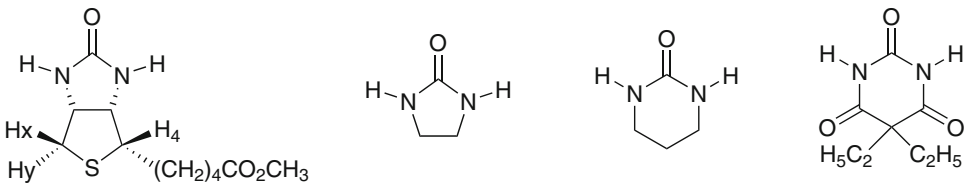
Guests are commercially available: biotin methyl ester (methylbiotin, **1**) (>99 %, dried under vacuum), 2-imidazolidinone (**2**) (96 %, recrystallized from ethyl acetate), *N*,*N*'- trimethyleneurea (**3**) (>98 %, recrystallized from ethyl acetate), and barbital (**4**) (>99 %). All starting reagents were obtained from commercial suppliers and used as received without further purification. Solvents were purified and dried with use of standard procedures. Compounds **5** [22], **6** [23] and **7** [23] were prepared according to literature. Melting points were determined with a Thermo-Galen hot stage microscope and are given uncorrected. Elemental analyses for carbon, hydrogen and nitrogen were performed by the Microanalytical Service of the Universidad Complutense of Madrid, using a Perkin-Elmer 240 analyzer. Mass spectrometry experiments were carried out on a Finnigan Surveyor (Thermo Electron, San José, CA, USA) pump coupled with a Finnigan LCQ Deca (Thermo Electron, San José, CA, USA) ion trap mass spectrometer using an electrospray ionization (ESI) interface. Methanolic solutions of the compounds (1.3 mg L^{-1}) were infused into the mass spectrometer at a flow rate of 5 μL min^{-1} using the syringe pump included in the LCQ instrument and mixing it with 100 μL min^{-1} of methanol:acetic acid 0.2 % (50:50, v/v) by means of a zero-dead volume T–piece. Mass spectra were acquired in positive mode, scanning from m/z 700 to m/z 2000. Spray voltage was set at 4.5 kV, heated capillary temperature at 200 °C, sheath gas at 0.6 L min^{-1} and, auxiliary gas at 6 L min^{-1}.

Synthesis

*N,N'-(7,7'-(2,16-bis(4-nitrophenylsulfonyl)-6,9,12-trioxo-2,16-diazaheptadecano-1,17-diyl)bis(1,8-naphthyridin-2,7-diyl))diacetamide (**9**)*

To a solution of *N*-(7-formyl-1,8-naphthyridin-2-yl)acetamide (**7**), 1.90 g (8.82 mmol), in 120 mL of methanol, 0.973 g (4.41 mmol) of 4,7,10-trioxatridecane-1,13-diamine were added. After 12 h at room temperature an aliquot of the reaction mixture was analyzed to verify that the starting material was consumed and the diimine formed. Then, slow addition of 0.7 g (17.64 mmol) of sodium borohydride was achieved. The reaction was maintained at room temperature with continuous stirring for 24 h and afterwards water was added to quench the excess $NaBH_4$.

Fig. 2 Selected guests **1–4**



The solvents were removed under vacuum and the crude residue extracted twice with dichloromethane/water. The organic fractions were combined, dried over anhydrous sodium sulfate and the solvent evaporated off to yield **8** as a brown hygroscopic oil (1.78 g) that was used without further purification.

Crude compound **8** was dissolved in 150 mL of dry dichoromethane (amylene stabilized) with 1.603 g (15.85 mmol) of triethylamine and was maintained at 0 °C using an ice bath. After addition of a solution of 1.75 g (8 mmol) of 4-nitrobenzenosulfonyl chloride in 50 mL of dichloromethane the reaction mixture was stirred at room temperature for 8 h and then extracted with water and 1 M hydrochloric acid. Organic layers were combined and the solvent evaporated off to yield a brown solid (2.3 g, 81 %), the diacetyl derivative **9**, which was purified by column chromatography over silica gel 60 mesh with dichloromethane/methanol (98:2 in volume). During these operations partial deacetylation occurred (0.2 g of **10)** and only 0.7 g of **9** were obtained: m.p. 142–145 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 8.94 (br s, 2H, CONH), 8.52 (d, 2 H, $^3J = 8.8$ Hz, 3′-H), 8.32 (d, 4 H, $^3J = 8.9$ Hz, *meta*-H), 8.17 (d, 2 H, $^3J = 8.8$ Hz, 4′-H), 8.14 (d, 2 H, $^3J = 8.2$ Hz, 5′-H), 8.05 (d, 4 H, $J = 8.8$ Hz, *ortho*-H), 7.63 (d, 2 H, $^3J = 8.2$ Hz, 6′-H), 4.70 (s, 4 H, Ar-CH_2NNs), 3.40 (t, 4H, $^3J = 7.5$ Hz, $OCH_2CH_2C\underline{H}_2N$), 3.35 (m, 8H, $OC\underline{H}_2C\underline{H}_2O$), 3.29 (t, 4 H, $^3J = 5.9$ Hz, $OC\underline{H}_2CH_2CH_2N$), 2.27 (s, 6 H, $C\underline{H}_3$-CONH), 1.87-1.49 (m, 4H, $OCH_2C\underline{H}_2CH_2N$); ^{13}C NMR ($CDCl_3$) δ (ppm) 169.7 (CO), 160.7 (C7′), 154.2 (C2′ or C8a'), 154.1 (C8a' or C2′), 150.1 (C_{para}), 145.5 (C_{ipso}), 139.4 (C4′), 137.6 (C5′), 128.6 (C_{ortho}), 124.5 (C_{meta}), 119.8 (C4a'), 119.7 (C6′), 115.5 (C3′), 70.5 and 70.3 ($OC\underline{H}_2C\underline{H}_2O$), 67.9 ($OC\underline{H}_2CH_2CH_2N$), 54.4 (Ar-$CH_2$NNs), 46.8 ($OCH_2CH_2$-$C\underline{H}_2N$), 28.5 ($OCH_2C\underline{H}_2CH_2N$), 25.1 ($C\underline{H}_3CO$). Anal. Calcd for $C_{44}H_{48}N_{10}O_{13}S_2.H_2O$: C, 53.43; H, 4.89; N, 14.16, S, 6.48 Found: C, 53.10; H, 5.25; N, 14.10; S, 6.35.

*N,N'-(3,3′-(2,2′-oxybis(ethane-1,2-diyl)bis(oxy))bis(propane-1,3-diyl))bis(N-((7-amino-1,8-naphthyridin-2-yl)methyl))-4-nitrobenzenesulfonamide (**10**)*

To a solution of 0.53 g (0.95 mmol) of **9** in 10 mL of dioxane and 2 mL of water, 1 mL of 4 M HCl in dioxane was added and the mixture heated for 30 min at 110 °C. The reaction mixture was cooled down and neutralized with 2.5 M sodium hydroxide to pH 8–9. The dioxane was evaporated under vacuum and the oily residue extracted with dichloromethane to yield **10** (0.45 g, 93 %): m.p. 102–105 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 8.26 (d, 4 H, $^3J = 8.8$ Hz, *meta*-H), 7.98 (d, 4 H, $^3J = 8.8$ Hz, *ortho*-H), 7.92 (d, 2 H, $^3J = 8.2$, 5′-H), 7.82 (d, 2 H, $^3J = 8.8$ Hz, 4′-H,), 7.37 (d, 2 H, $^3J = 8.2$ Hz, 6′-H,), 6.75 (d, 2 H, $^3J = 8.8$ Hz, 3′-H), 5.20 (s, 4 H, NH_2), 4.63 (s, 4 H, Ar-CH_2NNs), 3.54 (t, 4 H, $^3J = 7.5$ Hz, $OCH_2CH_2C\underline{H}_2N$), 3.42 (m, 8 H, $OC\underline{H}_2C\underline{H}_2O$), 3.32 (4 H, t, $^3J = 5.9$ Hz, $OC\underline{H}_2CH_2CH_2N$), 1.87-1.49 (m, 4 H, $OCH_2C\underline{H}_2CH_2N$); ^{13}C NMR ($CDCl_3$) δ (ppm) 160.0 (C7′), 159.3 (C2′), 155.8 (C8a'), 150.1 (C_{para}), 145.8 (C_{ipso}), 138.3 (C4′), 137.4 (C5′), 128.6 (C_{ortho}), 124.2 (C_{meta}), 117.4 (C6′), 116.5 (C4a'), 112.6 (C3′), 70.6 and 70.2 ($OC\underline{H}_2C\underline{H}_2O$), 68.1 ($OC\underline{H}_2CH_2CH_2N$), 54.4 (Ar-$CH_2$NNs), 47.1 ($OCH_2CH_2$-$C\underline{H}_2N$), 28.6 ($OCH_2C\underline{H}_2CH_2N$). Anal. Calcd for $C_{40}H_{44}$-$N_{10}O_{11}S_2$: C, 53.09; H, 4.90; N, 14.48; S,7.09 Found: C, 52.94; H, 4.82; N, 14.38; S, 7.01.

*Macrocycle **Ia***

0.83 g (0.92 mmol) of **10** and 0.376 g (3.67 mmol) of triethylamine were dissolved in 50 mL of dry chloroform. 0.187 g (0.92 mmol) of isophthaloyl dichloride was also dissolved in 50 mL of dry chloroform. In an oven dried 1 L three-necked round bottomed flask, maintained at 0 °C and under Argon atmosphere, containing 200 mL of dry chloroform, the two already prepared solutions were simultaneously added using independent pressure controlled addition funnels during a 2 h period. Then the reaction mixture was stirred for 48 h more at room temperature, the solvent was evaporated off and the crude purified by column chromatography over silica gel 60 mesh with dichloromethane-methanol (95.5 in volume) to afford **Ia** (200 mg, 21 %): m.p. > 250 °C (dec.); ^{1}H NMR ($CDCl_3$) δ (ppm) 9.57 (br. s, 2 H, CONH), 8.71 (d, 2 H, $^3J_{4'\text{-H}} = 8.8$ Hz, 3′-H), 8.62 (s, 1 H, 1-H), 8.39 (d, 4 H, $^3J_{ortho\text{-H}} = 8.8$ Hz, *meta*-H), 8.37 (d, 2H, $^3J_{4\text{-H}} = 7.8$ Hz, 3-H/5-H), 8.26 (d, 2 H, 4′-H), 8.16 (d, 2 H, $^3J_{6'\text{-H}} = 8.2$ Hz, 5′-H), 8.04 (d, 4 H, *ortho*-H), 7.77 (t, 1 H, 4-H), 7.58 (d, 2 H, 6′-H), 4.78 (s, 4 H, 9′-H), 3.54 (t, 4 H, $^3J_{12'\text{-H}} = 7.5$ Hz, 11′-H), 3.45 (m, 8 H, 14′-H, 15′-H),

3.37 (t, 4 H, $^{3}J_{12'\text{-H}} = 5.9$ Hz, 13′-H), 1.75 (m, 4 H, 12′-H); ^{13}C NMR ($CDCl_3$) δ (ppm) 164.8 (CO), 160.6 (C7′), 154.0 (C2′, C8a'), 150.1 (C_{para}), 145.4 (C_{ipso}), 139.6 (C4′), 137.5 (C5′), 134.0 (C2/C6), 133.2 (C3/C5), 130.4 (C4), 128.5 (C_{ortho}), 124.6 (C_{meta}), 124.3 (v. br. s, C1), 119.8 (C4a'), 119.7 (C6′), 115.2 (C3′), 70.6 (C14′ or C15′), 70.2 (C15′ or C14′), 68.1 (C13′), 53.8 (C9′), 47.0 (C11′), 28.9 (C12′); ^{15}N NMR ($CDCl_3$) δ (ppm) -79.5 (N8′), -109.5 (N1′); Anal. Calcd for $C_{48}H_{46}N_{10}O_{13}S_2\,H_2O$: C, 54.75; H, 4.48; N, 13.30, S, 6.09; Found: C, 54.27; H, 4.50; N, 13.14; S, 6.0; ESI–MS [M + H] 1035.2.

Macrocycle ***Ib***

0.83 g (0.92 mmol) of **10** and 0.376 g (3.67 mmol) of triethylamine were dissolved in 50 mL of dry chloroform. 0.188 g (0.92 mmol) of 2,6-pyridinedicarbonyl dichloride were also dissolved in 50 mL of dry chloroform. In an oven dried 1 L three-necked round bottomed flask, maintained at 0 °C and under Argon atmosphere, containing 200 mL of dry chloroform the two already prepared solutions were simultaneously added using independent pressure controlled addition funnels during a 2 h period. Then the reaction mixture was stirred for 48 h more at room temperature, the solvent was evaporated off and the crude purified by column chromatography over silica gel 60 mesh with dichloromethane-methanol (95:5) to afford **Ib** (100 mg, 10.5 %): m.p. ≥ 250 °C (dec); ^{1}H NMR ($CDCl_3$) δ (ppm) 11.24 (br. s, 2 H, CONH), 8.88 (d, 2 H, $^{3}J_{4'\text{-H}} = 8.8$ Hz, 3′-H), 8.64 (d, 2 H, $^{3}J_{4\text{-H}} = 7.7$ Hz, 3-H/5-H), 8.43 (m, 4 H, *meta*-H), 8.28 (d, 2 H, 4′-H), 8.25 (t, 1 H, 4-H), 8.17 (d, 2 H, $^{3}J_{6'\text{-H}} = 8.2$ Hz, 5′-H), 8.16 (m, 4 H, *ortho*-H), 7.57 (d, 2 H, 6′-H), 4.93 (s, 4 H, 9′-H), 3.65 (t, 4 H, $^{3}J_{12'\text{-H}} = 7.7$ Hz, 11′-H), 3.46 (m, 8 H, 14′-H, 15′-H), 3.42 (t, 4 H, $^{3}J_{12'\text{-H}} = 6.1$ Hz, 13′-H), 1.80 (m, 4 H, 12′-H); ^{13}C NMR ($CDCl_3$) δ (ppm) 162.4 (CO), 160.7 (C7′), 153.9 (C2′ or C8a'), 153.8 (C8a' or C2′), 150.1 (C_{para}), 148.4 (C2/C6), 145.2 (C_{ipso}), 139.7 (C4′), 139.6 (C4), 128.8 (C_{ortho}), 124.7 (C_{meta}), 126.6 (C3/C5), 119.7 (C4a'), 119.4 (C6′), 115.4 (C3′), 70.9 (C14′ or C15′), 70.3 (C15′ or C14′), 68.3 (C13′), 53.9 (C9′), 47.7 (C11′), 29.6 (C12′); ^{15}N NMR ($CDCl_3$) δ (ppm) -78.8 (N8′), -246.5 ($^{1}J_{NH} = 91.8$ Hz, HNCO); Anal. Calcd for $C_{47}H_{45}N_{11}O_{13}S_2\,3H_2O$: C, 51.78; H, 4.36; N, 14.13; S, 5.88; Found: C, 51.43; H, 4.36; N, 13.97; S, 5.80; ESI–MS [M + H] 1036.2.

Nuclear magnetic resonance studies

NMR spectra were recorded at 300 K (9.4 Tesla, 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C and 40.56 MHz for ^{15}N) with a 5-mm inverse-detection H–X probe equipped with a z-gradient coil. Chemical shifts (δ in ppm) are given from internal solvent $CDCl_3$ 7.26 for ^{1}H and 77.0 for ^{13}C and for ^{15}N NMR nitromethane was used as external standard. 2D experiments, gs-HMQC (^{1}H-^{13}C), gs-HMBC (^{1}H-^{13}C), gs-HMQC (^{1}H-^{15}N) and gs-HMBC (^{1}H-^{15}N), were carried out with the standard pulse sequences [24] to assign the ^{1}H, ^{13}C and ^{15}N signals. 2D NOESY spectra were acquired in the phase sensitive mode using mixing time of 850 ms (**Ia**) and 650 ms (**Ia**:**1**).

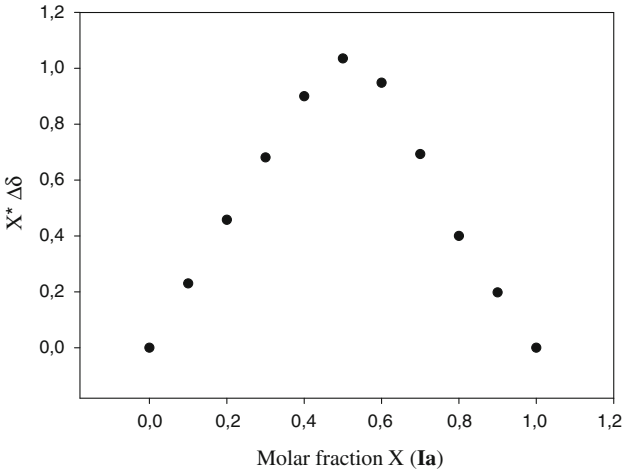


Fig. 3 Job plot titration for **Ia:1** (2 mM)

Determination of the stoichiometry by Job plot titrations

The stoichiometry of the host–guest complexes was determined by the continuous variation method [25–27]. Both stock solutions of the guests **1**–**4** (3 mM) and receptors **Ia** and **Ib** (3 mM) were prepared in a n/m (% v/v) mixture of $CDCl_3$. To obtain the desired host–guest ratios, which varied from 0 to 1 (molar fraction of **Ia**), appropriate volumes of stock solutions were mixed. In a final volume equal to 1,000 µl, the sum of guest and host concentrations is constant (3 mM). From the value of the maximum, which can be obtained by means of equation X = m/(m + n), the stoichiometry of the complex is determined. A representative curve for complex **Ia:1** is shown in Fig. 3.

Quantification of the binding constants K_b

Each NMR titration was carried out at least three times at 300 K in $CDCl_3$ (Merck S33657, deuterium content >99.8 %, water content <0.01 %). eVol® XR hand-held automated analytical syringes (500 µL, 50 µL) from SGE Analytical Sciences previously calibrated for $CDCl_3$, were used to perform additions. Host and guest were weighted in a Metler AE260-Delta Range scale (error ±0.00005 g). ^{1}H NMR titrations were used to quantify K_b values, These titrations were carried out monitoring the chemical induced shift (CIS) in one or more host or guest protons as the

concentration of the formed complex varies upon addition of one of the components. The CIS for amide protons while guest solution aliquots were added was monitored. There is a large number of ways to fit data from a titration [28] but that consisting in non-linear curve fitting is generally accepted as the method with the lowest error in the determination of K_b values, in comparison to others that employ approximations to reach a linear relationship between and K_b. Binding curves were fitted using Wineqnmr software [29]. The quality of the fit was estimated using the merit-function shown in Eq. 1 where w_i is the weight attributed to observation i (normally data points were assigned equal weights):

$$R = 100 \times \left\{ \left[\sum w_i (\delta_{obs} - \delta_{calc})^2 \right] / \sum w_i (\delta_{obs})^2 \right\}^{1/2} \quad (1)$$

the basic equation for this kind of titrations is represented in Eq. 2, showing the relationship between chemical shifts (δ), concentrations of host H, guest G and complex C, and the binding constant $K_b = [HG]/([H][G])$. This equation is valid only for 1:1 stoichiometry.

$$\Delta\delta = \delta_{HG}\left([HG]/[H]_o\right) \quad (2)$$

Crystal structure determination

Single crystals of $C_{49}H_{51}N_{11}O_{14}S_3$ (**Ib**) were grown from dimethylsulfoxide-water. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil on a Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 100.15 K during data collection. Using Olex2 [30], the structure was solved with the ShelXS [31] structure solution program using direct methods and refined with the ShelXL [31] refinement package using least squares minimization.

Crystal data for $C_{49}H_{51}N_{11}O_{14}S_3$ (M = 1114.18): triclinic, space group P-1 (No. 2), a = 11.0495(3) Å, b = 12.5732(4) Å, c = 18.6966(5) Å, α = 85.817(2)°, β = 79.788(2)°, γ = 84.750(2)°, V = 2541.34(12) Å^3, Z = 2, T = 100.15 K, μ(Cu Kα) = 2.007 mm^{-1}, D*calc* = 1.456 mg/mm^3, 45532 reflections measured (7.072 ≤ 2Θ ≤ 124.114), 7955 unique (R_{int} = 0.0271) which were used in all calculations. The final R_1 was 0.0347 ($I > 2\sigma(I)$) and wR_2 was 0.0991 (all data). CCDC reference number is CCDC-951726.

Molecular modeling

MacroModel v.8.1, with the GB/SA model for chloroform was used to perform the molecular simulations of hosts, guests and complexes [32]. All calculations were achieved with Montecarlo (MC) conformational analyses [33]. Minimization is carried out using Polak-Ribiere Conjugate Gradient (PRCG) optimizer [34, 35] as implemented in the program version, the energy gradient was chosen as the convergence criteria with a value of 0.05, and at least 2,000 iterations. All MC calculations were performed with Montecarlo multiple minimum (MCMM) method, and the variables were torsion angles, molecule coordinates or both. The minimization method was PRCG with the same characteristics described above. In a typical MC run a MCMM is never performed with less than 8,000 steps, to carry out the search both torsional rotations in host and guest and translation/rotation (10 Å/360°) of the guest is performed, for all the MC a cutoff is applied to van der Waals, electrostatic and H-bond interactions with 7, 12 and 4 Å respectively. These calculations were carried out with the AMBER* force field [36] as implemented in the version of the program.

Results and discussion

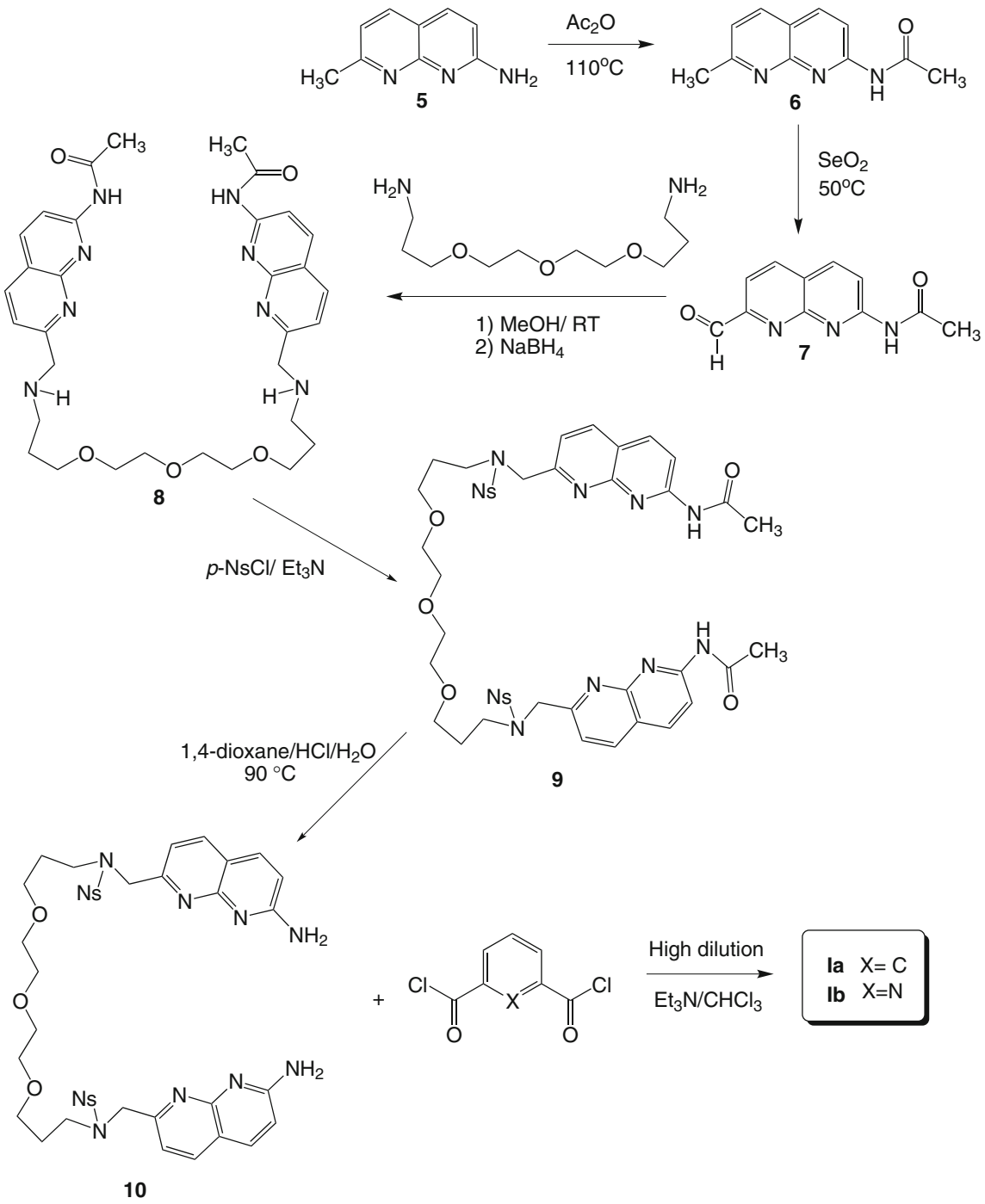
Receptors studies

Synthesis

Macrocycles **Ia** and **Ib** were synthesized according to Scheme 1, starting from 2-amino-7-methyl-1,8-naphthyridine (**5**) [22], which was first acetylated to yield **6** and later its methyl group oxidized to afford the corresponding aldehyde **7** [23]. Condensation of two equivalents of **7** with 4,7,10-trioxatridecane-1,13-diamine in methanol and subsequent reduction of the imine groups with sodium borohydride gave *N,N'*-(7,7′-(6,9,12-trioxa-2,16-diazaheptadecane-1,17-diyl)-bis(1,8-naphthyridine-2,7-diyl))diacetamide (**8**). The crude product was treated first with 4-nitrobenzenesulfonyl chloride (*p*-nosyl chloride, NsCl) and after with hydrochloric acid in dioxane-water to yield (**10**). Reaction of **10** with isophthaloyl dichloride or 2,6-pyridinedicarbonyl dichloride using triethylamine and chloroform in high dilution conditions gave the macrocyclic receptors **Ia** and **Ib**, respectively.

Nuclear magnetic resonance studies

A complete characterization of the macrocycles was carried out by ^{1}H, ^{13}C and ^{15}N NMR spectroscopy in $CDCl_3$ as solvent. For symmetry reasons, besides the isochronous atoms in the central bisamido ring, benzene and pyridine (3-H/5-H, C2/C6 and C3/C5), these molecules show equivalent naphthyridinyl and methylene groups. Full assignment of protons and carbons was achieved by analysis of the chemical shift values, multiplicity of the signals and the coupling constants magnitude, as well as gs-COSY, gs-HMQC and gs-HMBC bidimensional experiments. The ^{15}N-NMR chemical shifts were assigned using gs-HMQC



Scheme 1 Synthetic procedure used to obtain macrocycles **Ia** and **Ib**

and gs-HMBC (^{1}H-^{15}N) correlation experiments, but only N1′ at −109.5 ppm and N8‘at −79.5 ppm in receptor **Ia,** and N8′ at −78.8 ppm and the NHCO at −246.5 ppm ($^{1}J_{NH} = 91.8$ Hz) in receptor **Ib,** were detected (See Electronic supplementary material).

The NH amide protons appear at 9.57 ppm in **Ia** and 11.24 ppm in **Ib**, the more deshielded value encountered in the macrocycle containing the 2,6-bisamidopyridine central core confirming that these protons are involved in an intramolecular hydrogen bond with the pyridine nitrogen atom, just as in the open receptors previously studied in our group [13]. Figures 4 and 5 show the ^{1}H NMR spectra of both macrocycles. The protons of the benzene or pyridine rings are easy to identify by integration and signal multiplicity. In **Ia**, the 1-H proton appears as a broad singlet at 8.62 ppm and the 4-H as a triplet, due to coupling with the

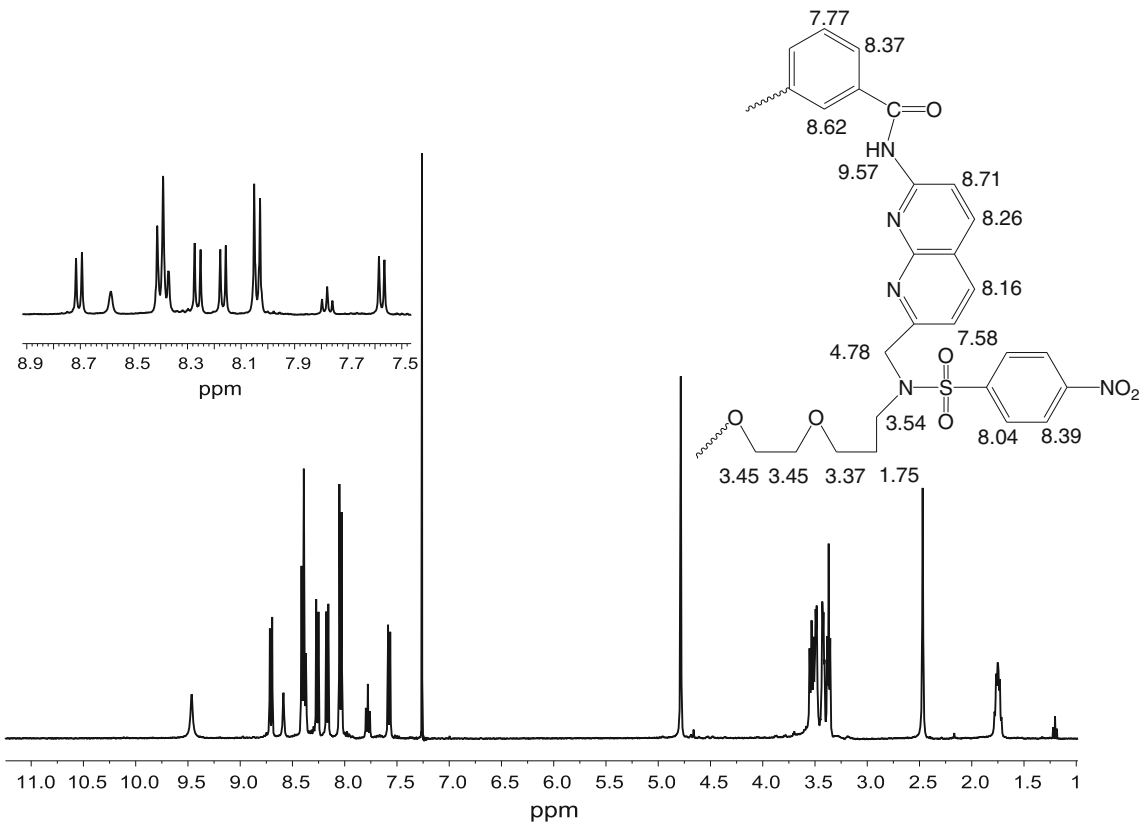


Fig. 4 ^{1}H NMR spectrum of macrocycle **Ia** in $CDCl_3$ (15 mM)

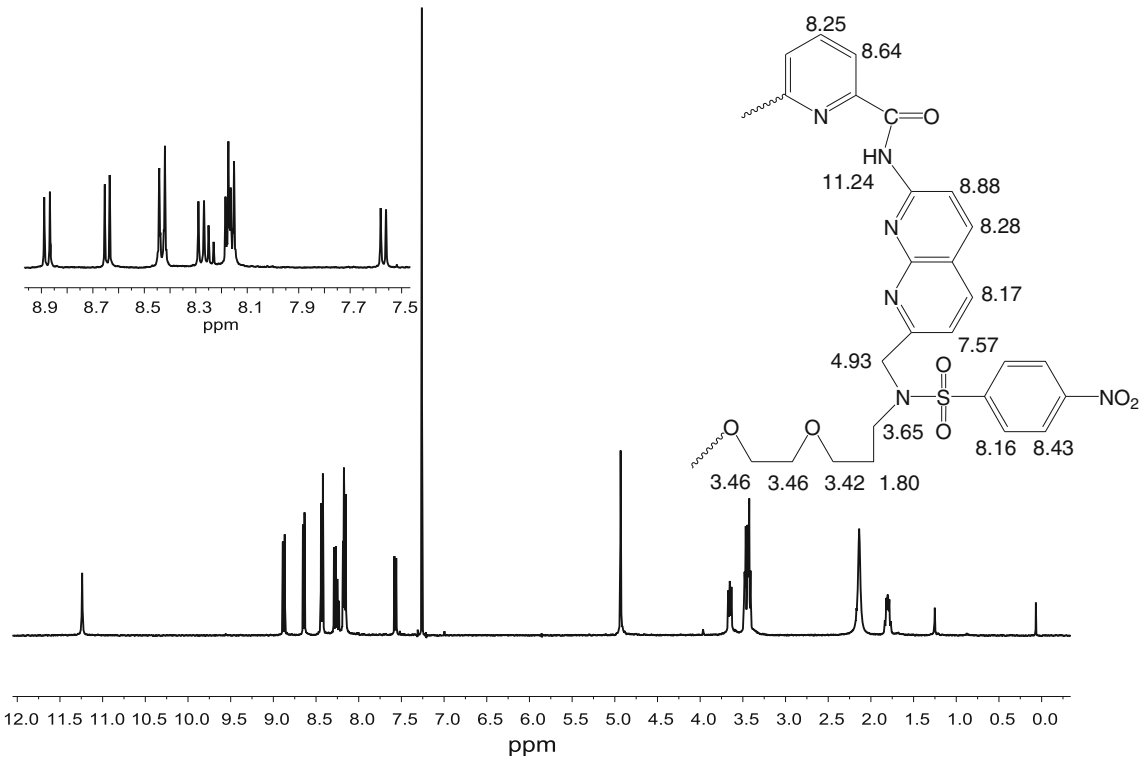


Fig. 5 ^{1}H NMR spectrum of macrocycle **Ib** in $CDCl_3$ (15 mM)

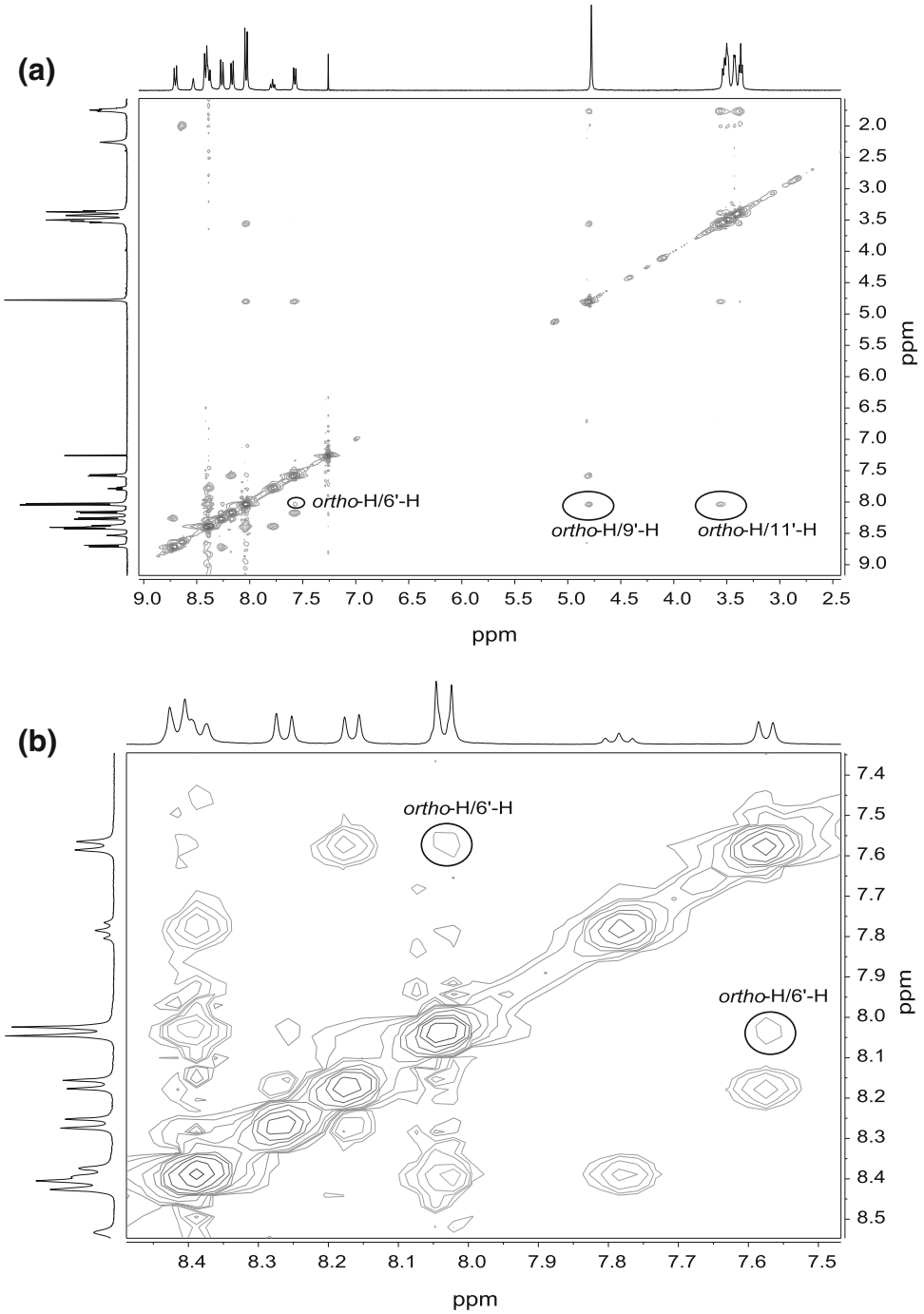


Fig. 6 2D NOESY spectrum of **Ia** in $CDCl_3$: **a** *ortho*-H with 6′-H, 9′-H and 11′-H correlations **b** enlarged region showing the *ortho*-H/6′-H correlation

magnetically equivalent 3-H and 5-H with a coupling constant of 7.8 Hz. The doublet signal at 8.37 ppm corresponds to the 3-H and 5-H protons. In **Ib**, the signals of equivalent pyridine protons 3-H and 5-H appear as a doublet with a coupling constant of 7.7 Hz with 4-H at 8.25 ppm. In both receptors, ^{1}H-NMR chemical shifts of the naphthyridine unit follow the order: 3′-H > 4′-H > 5′-H > 6′-H and appear as doublet signals in the range of 7.57–8.88 ppm with ^3J (3′-H, 4′-H) of 8.8. Hz and ^3J (5′-H, 6′-H) of 8.2 Hz.

For the methylene groups of the ether chain, the most deshielded signals correspond to 9′-H (singlet) and 11′-H (triplet) protons because their proximity to the electron-withdrawing sulfonamide group. The values of chemical shifts follow the order: 9′-H > 11′-H > 14′-H = 15′-H > 13′-H > 12′-H.

The 2D NOESY experiment carried out using a 15 mM solution of host **Ia** in $CDCl_3$ shed some light on the spatial arrangement of the *p*-nosyl groups around the cavity (Fig. 6). On the basis of the following correlations, *ortho*-

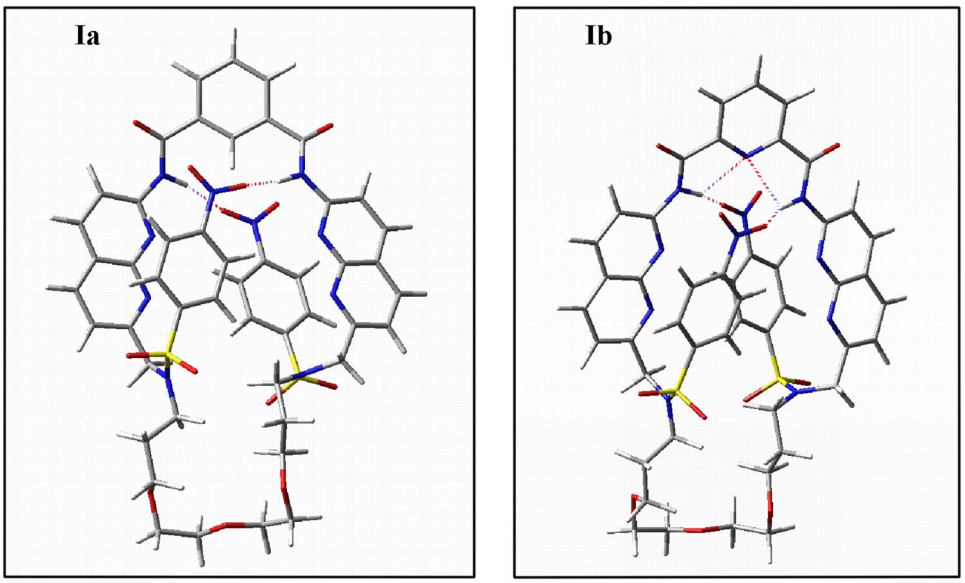


Fig. 7 Minimum energy conformations of macrocycles **Ia** and **Ib**

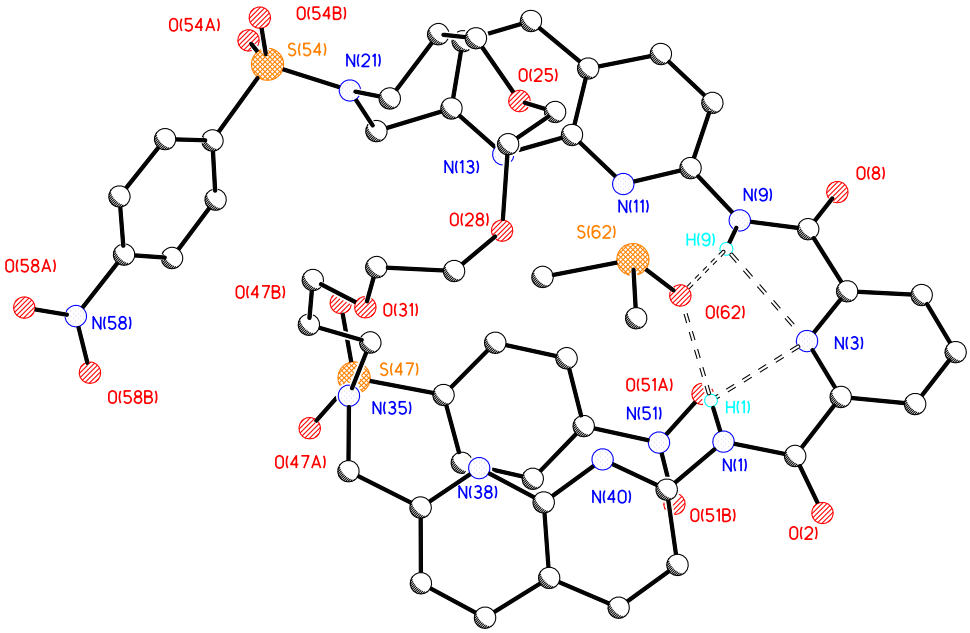


Fig. 8 X-ray crystal structure of **Ib** with heteroatom labeling, showing the H bonding interactions

H/9′-H, *ortho*- H/6′-H, and *ortho*- H/11′-H, we concluded that the *p*-nosyl groups are pointing upwards towards the HB (benzenedicarboxamide/naphthyridines) recognition site of the macrocycle.

Molecular modeling

The minimum energy conformations of macrocycles **Ia** and **Ib** were obtained from Monte Carlo conformational searches with AMBER force field (Fig. 7). In **Ia**, the nitro group of the two *p*-nosyl pendant arms is hydrogen bonded to each NH of the isophthaloyl amides forming a small aromatic box, in agreement with the experimental evidence found in the NOESY experiment discussed above (Fig. 6). In **Ib** these hydrogen bonds are also shared with the central pyridine nitrogen. The intramolecular HB network maintains the π-π stacking between the *p*-nosyl groups and the naphthyridine units.

X ray crystal structural analysis

We were able to obtain crystals of macrocycle 1b of suitable quality for single crystal Xray diffraction from dimethylsulfoxide-water and it crystallizes in the triclinic space group *P*-1 and the asymmetric unit contains the macrocycle ($C_{47}H_{45}N_{11}O_{13}S_2$) with a molecule of DMSO (Fig. 8).

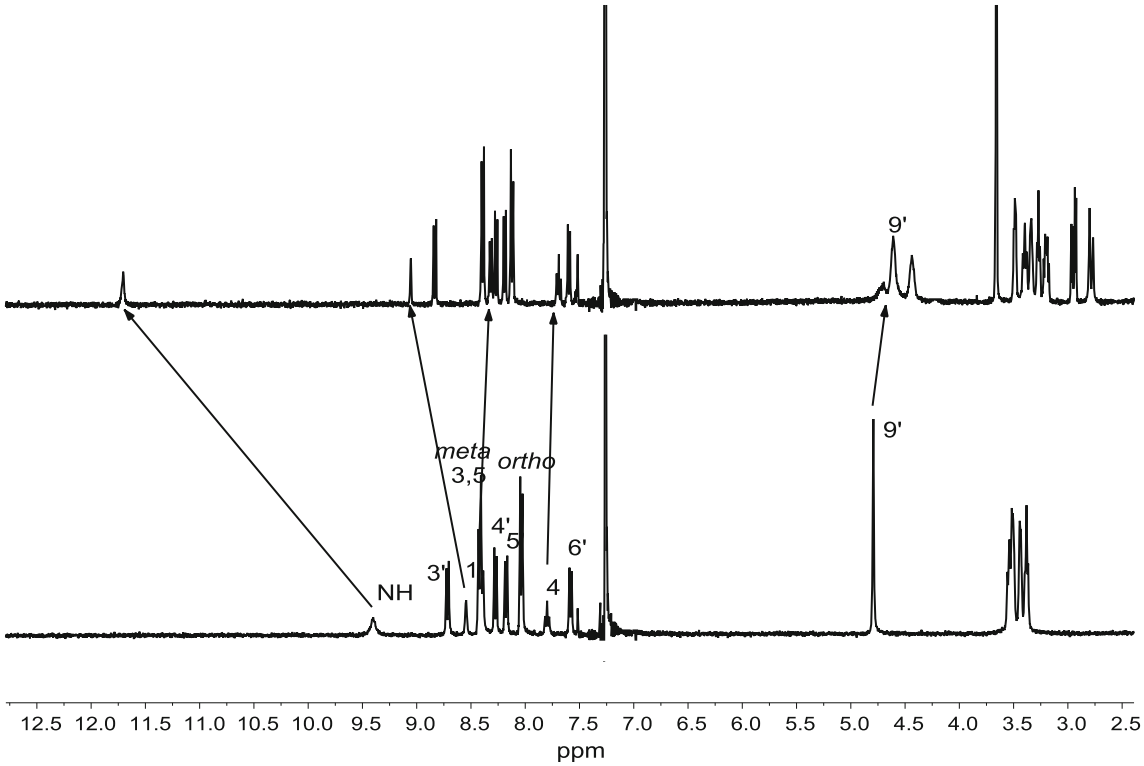


Fig. 9 ^{1}H NMR spectra of the macrocycle **Ia** (10^{-3} M in $CDCl_3$) at the starting point of the titration (*bottom*) and when 1.2 equiv of guest **1** have been added (*top*)

Table 1 Hydrogen bonding (Å and °) with esds (except fixed and riding H)

D-H···A	d(D-H)	d(H···A)	d(D···A)	<(DHA)
Contacts from NHs of the pyridine carboxamides				
N1-H1···O6	0.81(2)	2.07(3)	2.852(2)	164(2)
N9-H9···O62	0.81(2)	2.01(2)	2.803(2)	164(2)
Intra-contacts from NHs of the pyridine carboxamides to the pyridine N				
N1-H1···N3	0.81(2)	2.36(2)	2.704(2)	107(2)
N9-H9···N3	0.81(2)	2.30(2)	2.695(2)	110.6(19)
Contacts from methyls C61 and C63 to the ether chain				
C63-H63B···O25	0.98	3.10	3.561(2)	110.4
C63-H63B···O31A_b	0.98	3.00	3.824(4)	142.3
C61-H61C···O28_a	0.98	2.39	3.296(10)	152.8
C61-H61C···O28A_b	0.98	2.84	3.722(6)	149.5
C63-H63B···O28_a	0.98	2.59	3.452(13)	146.2
C63-H63B···O28A_b	0.98	2.78	3.671(8)	151.6
Contacts from methyls C61 and C63 to naphthyridine Ns				
C63-H63A···N11	0.98	2.92	3.626(2)	129.7
C63-H63A···N13	0.98	2.71	3.683(2)	172.2
C63-H63C···N38	0.98	2.87	3.821(2)	164.0
C63-H63C···N40	0.98	2.77	3.641(2)	147.7

The DMSO molecule is chelated by Hunter style [37] bifurcated hydrogen bonds NH···O to the biscarboxamides and by several longer contacts between the DMSO methyl groups C61 and C63 and ether chain oxygens and naphthyridine nitrogens of the macrocycle (Fig. 8).

These and other close contacts are tabulated in Table 1. Internal π-π stacking between one of the nosyl groups and a naphthyridine unit [closest atomic contact N38-C48 of 3.08 Å], and the 2,6-pyridinebiscarboxamide and a naphthyridine [closets atomic contact C4-C18 of

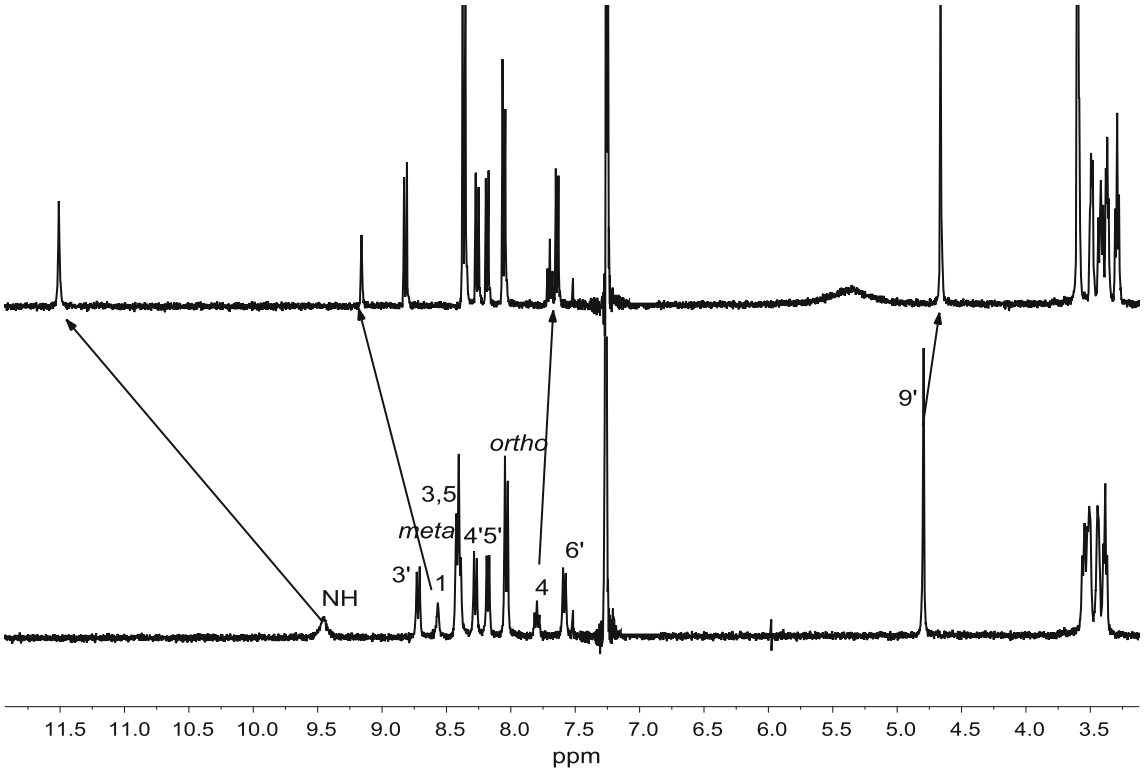


Fig. 10 ^{1}H NMR spectra of the macrocycle **Ia** (7×10^{-4} M in $CDCl_3$) at the starting point of the titration (*bottom*) and when 5 equiv of guest **2** have been added (*top*)

3.33 Å] are also observed in the solid state structure of macrocycle **Ib**.

Host–guest properties

Binding constant quantification

^{1}H NMR titrations in $CDCl_3$ at 300 K have been performed to quantify the interactions between receptors **Ia** and **Ib** and guests **1**–**4**. Before the quantification of the binding constants, the stoichiometry of the complexes was determined by using the method of continuous variation to generate Job plots and 1:1 stoichiometries for all complexes were obtained.

The host–guest binding constants (K_b) have been measured using the CIS on the NH signal of the amide groups of the receptors. In Figs. 9 and 10 we present the ^{1}H NMR spectra in the titrations of complexes **Ia**:**1** and **Ia**:**2**. The complete set of titrations for all complexes can be found in the Electronic supplementary material.

In macrocycle **Ia**, the aromatic protons 3-H and 5-H of the isophthaloyl ring are shielded about 0.1 ppm upon complexation of **1**, whereas such effect is not observed with the other guests (*c. f.* **Ia**: **2** in Fig. 10), indicating a conformational change of **Ia**. Another interesting feature is the fact that 9′-H proton signals broaden and appear at around 4.7 ppm in the region of 3a-H and 6a-H of biotin methyl ester **1**. Aliphatic protons of **Ia** and also the C5 chain protons of biotin show some deshielding due to their exposure to the rich π environment of the inner cavity of the macrocycle. In the 2D NOESY spectra of the **Ia**:**1** complex, the 9′-H protons are correlated with Hx and 4-H protons of the biotin ring, suggesting that the ureido part of the latter sits in the central cavity of the macrocycle, and *ortho*-H of the *p*-nosyl groups show correlation with 6′-H of the naphthyridine.

Table 2 Experimental binding constants K_b (M^{-1}) at 300 K for the complexes

Guest	**Ia**	**Ib**
1	18,000 ± 1,600	230 ± 30
2	6,000 ± 300	162 ± 40
3	8,000 ± 600	120 ± 20
4	800 ± 70	70 ± 7

The experimental binding constants K_b (M^{-1}) of receptors **Ia** and **Ib** with the four guests **1**–**4** are gathered in Table 2. Examination of the data indicates that the stability constants are moderate ranging over 3 orders of magnitude from **Ia**:**1** (1.8×10^4) to **Ib**:**4** (7.0×10). For all guests, **Ia**

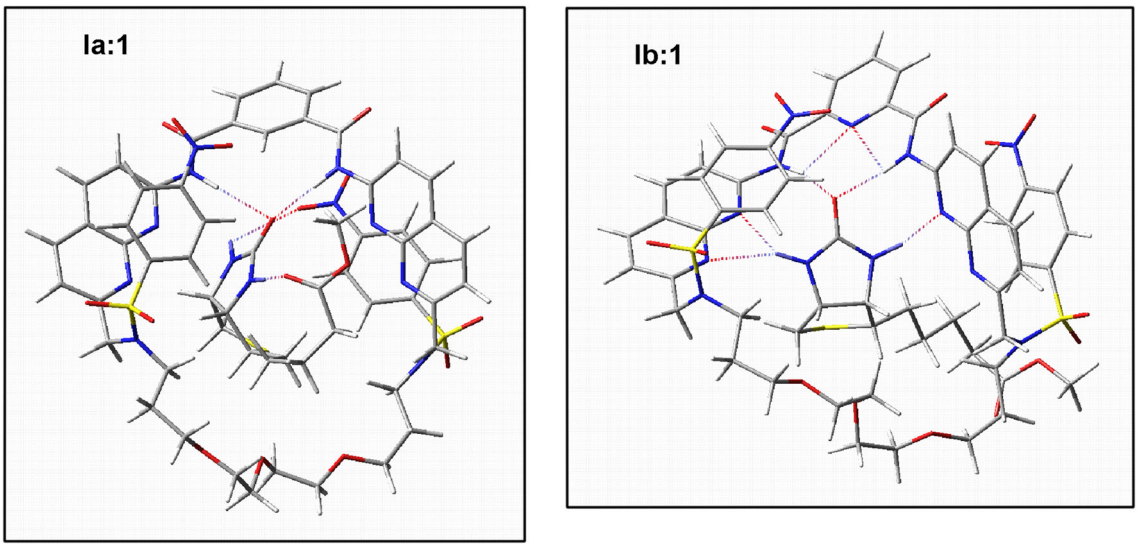


Fig. 11 Minimum energy conformations of complexes between **Ia** and **Ib** with (+)-biotin methyl ester (**1**)

has proved to be a better receptor than **Ib** by 2 orders of magnitude, and no significant improvement has been found with respect to known open receptors [11–13].

Molecular modeling

In the same manner as we did for the macrocyclic receptors, all complexes were modeled using Monte Carlo conformational search with the AMBER force field. The calculated enthalpies [$E_{interaction} = E_{min}$ (complex)–E_{min} (receptor)–E_{min} (guest), kJ mol^{-1}] pointed out the following relative stability order, **Ia**:**1** (−57.7) > **Ia**:**3** −41.6) > **Ia**:**2** −36.6) and **Ib**:**1** (−49.7) > **Ib**:**3** (−40.0) ∼ **Ib**:**2** (−39.8), which agree with the experimental K_b values (free energies ΔG) of Table 2 only in a qualitative way, most probably because entropy variation is not the same for all cases.

The calculated enthalpies obtained for complexes involving barbital **Ia**:**4** (−58.3) and **Ib**:**4** (−82.6) cannot be compared with the previous ones. Its complexation mode is quite different, involving the carbonyl amide group close to the ethyl substituent and not the ureido motif.

We reproduce in Fig. 11 the geometries obtained for complexes **Ia**:**1** and **Ib**:**1**, showing the hydrogen bonds that held guest **1** into the macrocycles and the two aromatic *p*-nosyl arms maintaining the π-π stacking with the naphthyridine rings. Interesting to note that in **Ia**:**1** complex, guest **1** presents a folded side chain, the carboxyl group of the ester forming an additional intramolecular HB with one of the ureido NHs. The increased preorganization via intramolecular HB in host **Ib** decreases the flexibility needed to accommodate the guests inside the cavity, and would account for the lower binding constants obtained for the complexes involving this macrocycle.

Conclusions

We report here the design and synthesis of two new macrocyclic receptors **Ia** and **Ib** and their molecular recognition studies for methyl biotin ester, 2-imidazolidinone, trimethylene urea and barbital. The binding constant values determined by ^{1}H NMR titration and the best results are obtained for methyl biotin ester and the isophthaloyl bisamido naphthyridine **Ia**. Molecular mechanics calculations account for the experimental binding constants only in a qualitative way due to the fact that these modeling studies do not take into account entropic factors or solvation-desolvation effects [38, 39]. In our previous papers, the entropy changes were the same or rather close for all series of complexes and good results were obtained in discussing the geometries of the energetic minima.

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References

1. Monographs in Supramolecular Chemistry. 11 vols. Royal Society of Chemistry, London (2006–2013)
2. Livnah, O., Bayer, E.A., Wilchek, M., Sussman, J.L.: Three-dimensional structures of avidin and the avidin-biotin complex. Proc. Natl. Acad. Sci. USA. **90**, 5076–5080 (1993)
3. Young, T., Abel, R., Kim, B., Berne, B.J., Friesner, R.A.: Motifs for molecular recognition exploiting hydrophobic enclosure in protein-ligand binding. Proc. Natl. Acad. Sci. USA. **104**, 808–813 (2007)
4. Adrian Jr, J.C., Wilcox, C.S.: Chemistry of synthetic receptors and functional group arrays. 10. Orderly functional group dyads.

Recognition of biotin and adenine derivatives by a new synthetic host. J. Am. Chem. Soc. **111**, 8055–8057 (1989)
5. Goswami, S., Dey, S.: Directed molecular recognition: design and synthesis of neutral receptors for biotin to bind both its functional groups. J. Org. Chem. **71**, 7280–7287 (2006)
6. Claramunt, R.M., Herranz, F., Santa María, M.D., Pinilla, E., Torres, M.R., Elguero, J.: Molecular recognition of biotin, barbital and tolbutamide with new synthetic receptors. Tetrahedron **61**, 5089–5100 (2005)
7. Ghosh, K., Sen, T.: A benzothiazole-based simple receptor in fluorescence sensing of biotin ester and urea. Tetrahedron Lett. **50**, 4096–4100 (2009)
8. Murray, T.J., Zimmerman, S.C.: 7- Amido-1,8-naphthyridines as hydrogen bonding units for the complexation of guanine derivatives: the role of 2-alkoxyl groups in decreasing binding affinity. Tetrahedron Lett. **42**, 7627–7630 (1995)
9. Mayer, M.F., Nakashima, S., Zimmerman, S.C.: Synthesis of a soluble ureido-naphthyridine oligomer that self-associates via eight contiguous hydrogen bonds. Org. Lett. **7**, 3005–3008 (2005)
10. Hamilton, A.D., Pant, N.: Nucleotide base recognition: ditopic binding of guanine to a macrocyclic receptor containing naphthyridine and naphthalene units. Chem. Commun. **12**, 765–766 (1988)
11. Claramunt, R.M., Herranz, F., Santa María, M.D., Jaime, C., de Federico, M., Elguero, J.: Towards the design of host–guest complexes: biotin and urea derivatives versus artificial receptors. Biosens. Bioelectron. **20**, 1242–1249 (2004)
12. Herranz, F., Santa María, M.D., Claramunt, R.M.: Molecular recognition: improved binding of biotin derivatives with synthetic receptors. J. Org. Chem. **71**, 2944–2951 (2006)
13. Santa María, D., Farrán, M.A., García, M.A., Pinilla, E., Torres, M.R., Elguero, J., Claramunt, R.M.: Synthetic hosts for molecular recognition of ureas. J. Org. Chem. **76**, 6780–6788 (2011)
14. Ghosh, K., Sen, T.: Naphthyridine-based receptors for flurometric detection of urea and biotin. J. Incl. Phenom. Macrocycl. Chem. **67**, 271–280 (2010)
15. Petitjean, A., Cuccia, L.A., Lehn, J.M., Nierengarten, H., Schmutz, M.: Cation-promoted hierarchical formation of supramolecular assemblies of self-organized helical molecular components. Angew. Chem. Int. Ed. **41**, 1195–1198 (2002)
16. Katz, J.L., Geller, B.J., Foster, P.D.: Oxacalixarenes and oxacyclophanes containing 1,8-naphthyridines: a new class of molecular tweezers with concave-surface functionality. Chem. Comm. **10**, 1026–1028 (2007)
17. Petitjean, A., Cuccia, L.A., Schmutz, M., Lehn, J.M.: Naphthyridine-based helical foldamers and macrocycles: synthesis, cation binding, and supramolecular assemblies. J. Org. Chem. **73**, 2481–2495 (2008)
18. Sijbesma, R.P., Beijer, F.H., Brunsveld, L., Folmer, B.J.B., Hirschberg, J.H.K.K., Lange, R.F.M., Lowe, J.K.L., Meijer, E.W.: Reversible polymers formed from self-complementary monomers using quadruple hydrogen bonding. Science **278**, 1601–1604 (1997)
19. Park, T., Zimmerman, S.C.: Formation of a miscible supramolecular polymer blend through self-assembly mediated by a quadruply hydrogen-bonded heterocomplex. J. Am. Chem. Soc. **128**, 11582–11590 (2006)
20. Mazik, M., Sicking, W.: Molecular recognition of carbohydrates by artificial receptors: systematic studies towards recognition motifs for carbohydrates. Chem. Eur. J. **7**, 664–670 (2001)
21. Mazik, M., Cavga, H.: An acyclic aminonaphthyridine-based receptor for carbohydrate recognition: binding studies in competitive solvents. Eur. J. Org. Chem. 3633–3638 (2007)
22. Brown, E.V.: 1,8-Naphthyridines. I. Derivatives of 2-and 4-methyl-1,8-naphthyridines. J. Org. Chem. **30**, 1607–1610 (1965)
23. Goswami, S., Mukherjee, R., Mukherjee, R., Jana, S., Maity, A.C., Adak, A.K.: Simple and efficient synthesis of 2,7-difunctionalized-1,8-naphthyridines. Molecules **10**, 929–936 (2005)
24. Berger, S., Braun, S.: 200 and More NMR Experiments. Wiley, Weinheim (2004)
25. Job, P.: Formation and stability of inorganic complexes in solution. Ann. Chim. App. **9**, 113–203 (1928)
26. MacCarthy, P., Hill, Z.D.: Novel approach to Job's method. J. Chem. Ed. **63**, 162–167 (1986)
27. Renny, J.S., Tomasevich, L.L., Tallmadge, E.H., Collum, D.B.: Method of continuous variations: Applications of Job plots to the study of molecular associations in organometallic chemistry. Angew. Chem. Int. Ed. **52**, 2–18 (2013)
28. Thordarson, P.: Determining association constants from titration experiments in supramolecular chemistry. Chem. Soc. Rev. **40**, 1305–1323 (2011)
29. Hynes, M.J.: EQNMR: a computer program for the calculation of stability constants from nuclear magnetic resonance chemical shift data. J. Chem. Soc. Dalton Trans. 311–312 (1993)
30. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J.A.K., Puschmann, H.: OLEX2: a complete structure solution, refinement and analysis program. J. Appl. Cryst. **42**, 339–341 (2009)
31. Sheldrick, G.M.: A short history of SHELX. Acta Cryst. **A64**, 112–122 (2008)
32. Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T., Still, W.C.: MacroModel-an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. J. Comp. Chem. **11**, 440–467 (1990)
33. Chang, G., Guida, W.C., Still, W.C.: An internal-coordinate Monte Carlo method for searching conformational space. J. Am. Chem. Soc. **111**, 4379–4386 (1989)
34. Polak, E.: Computational methods in optimization. Academic Press, New York (1971)
35. Brodlie, K.W. In: The state of the art in numerical analysis. Jacobs, D.A.H. (ed.). Academic Press, London (1977)
36. Weiner, P.K., Kollmann, P.A.: AMBER: assisted model building with energy refinement. A general program for modeling molecules and their interactions. J. Comput. Chem. **2**, 287–303 (1981)
37. Carver, F.J., Hunter, C.A., Shannon, R.J.: Directed macrocyclization reactions. J. Chem. Soc. Chem. Comm. **10**, 1277–1280 (1994)
38. Cohen, N.C. (ed.): Guidebook on molecular modeling in drug design. Academic Press Ltd, London (1996)
39. Lewars, E.G.: Computational chemistry. Introduction to the theory and applications of molecular and quantum mechanics. Springer, New York (2011)