ORGANIC
LETTERS

2012
Vol. 14, No. 14
3580–3583

Synthesis of Enantiopure, Trisubstituted Cryptophane-A Derivatives

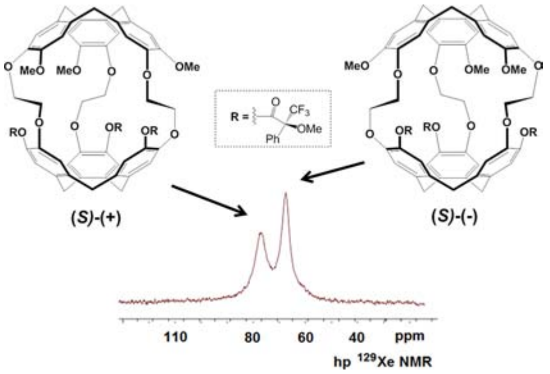
Olena Taratula, Michael P. Kim, Yubin Bai, John P. Philbin, Brittany A. Riggle, Danniebelle N. Haase, and Ivan J. Dmochowski*

Department of Chemistry, University of Pennsylvania, 231 South 34th Street,
Philadelphia, Pennsylvania 19104, United States

ivandmo@sas.upenn.edu

Received April 11, 2012

ABSTRACT



The efficient synthesis of enantiopure, trisubstituted cryptophane-A derivatives, organic host molecules with unusually high xenon affinity, is reported. Synthesis and chromatographic separation of (±) tri-Mosher's acid substituted cryptophane diastereomers gave ready access to the enantiopure cryptophanes, which are critical components in the design of enantiomerically pure ^{129}Xe biosensors. Hyperpolarized ^{129}Xe NMR spectroscopy identified single resonances for both trisubstituted cryptophane diastereomers that were separated by 9.5 ppm. This highlights opportunities for using enantiopure xenon biosensors in the simultaneous detection of ^{129}Xe in different biochemical environments.

The need for imaging agents and analytical tools that can report on the concentration and activity of various biomolecules in complex media has motivated the development of ^{129}Xe NMR biosensors.[1] These agents have the potential to detect cancer and other diseases by localizing hyperpolarized (hp) ^{129}Xe to a diseased tissue and/or by multiplexed detection of different protein biomarkers.[1] To date, cryptophane-A organic cages, in which two cyclotriguaiacylene (CTG) units are connected by three ethylene oxide linkers, show the highest xenon binding affinity with dissociation constants of ~25 μM at physiological temperature in aqueous solution.[1,2] Functionalized ^{129}Xe cryptophane biosensors can be targeted to different protein receptors and identified by changes to the frequency of the bound ^{129}Xe nucleus.[3]

The use of enantiopure cryptophanes is preferred over racemic mixtures, which have been shown to produce multiple, diastereomeric peaks upon binding to chiral protein surfaces.[4] Similarly complex hp ^{129}Xe NMR spectra are observed when racemic cryptophanes are modified with chiral small molecules or peptides, based on diastereomeric splitting.[5] For the sensitive detection of chiral biological analytes, enantiopure

---

(1) (a) Taratula, O.; Dmochowski, I. J. *Curr. Opin. Chem. Biol.* **2010**, *14*, 97. (b) Brotin, T.; Dutasta, J. P. *Chem. Rev.* **2009**, *109*, 88.

(2) Hill, P. A.; Wei, Q.; Eckenhoff, R. G.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2007**, *129*, 9262.

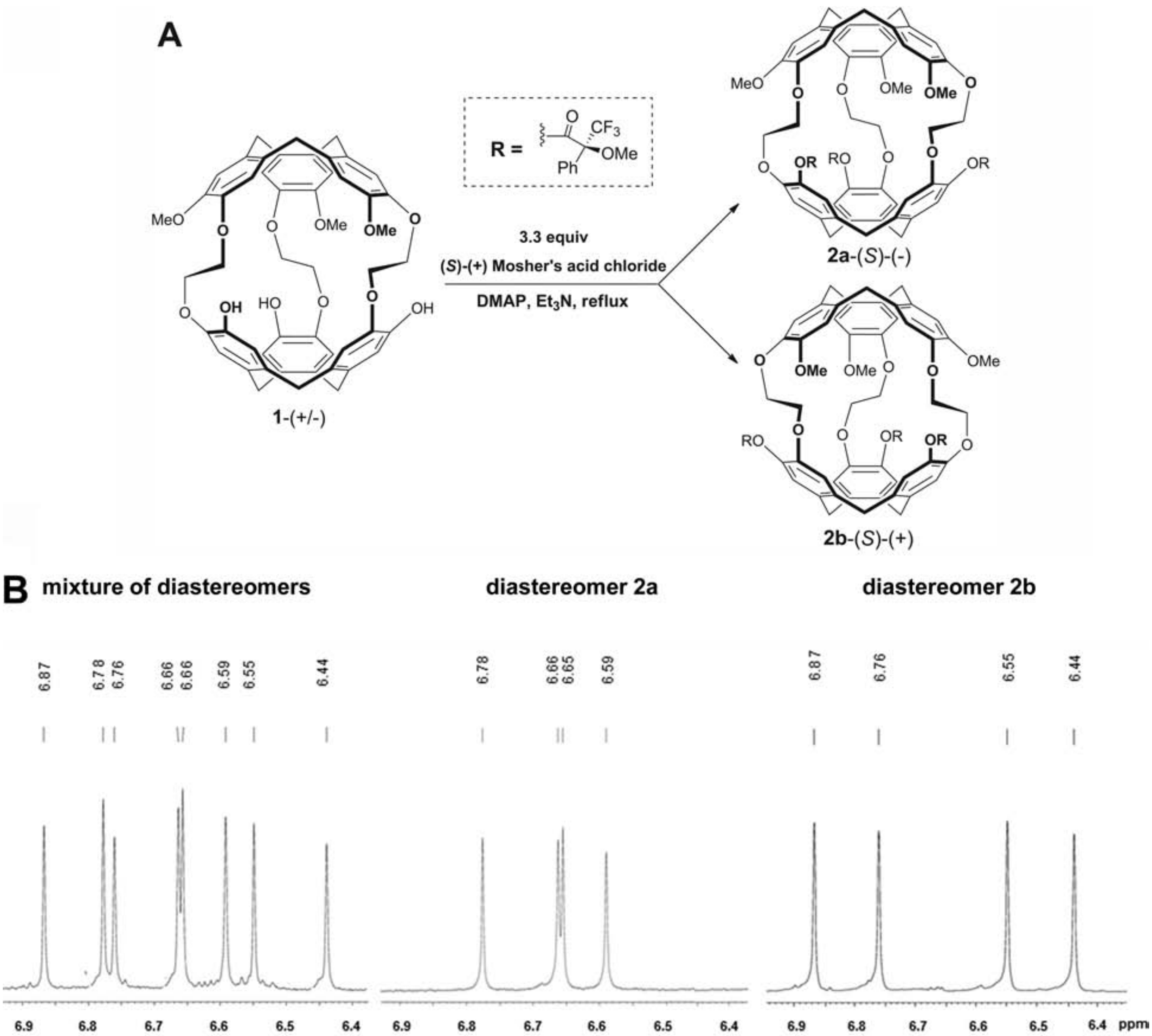
(3) (a) Berthault, P.; Roy, V.; Brotin, T.; Dutasta, J. P.; Charles, M. H.; Delair, T.; Mallet, F.; Huber, G.; Desvaux, H.; Boulard, Y. *ChemPhysChem* **2007**, *8*, 2082. (b) Freund, C.; Schlundt, A.; Kilian, W.; Beyermann, M.; Sticht, J.; Gunther, S.; Hopner, S.; Falk, K.; Roetzschke, O.; Mitschang, L. *Angew. Chem., Int. Ed.* **2009**, *48*, 4142. (c) Pines, A.; Spence, M. M.; Rubin, S. M.; Dimitrov, I. E.; Ruiz, E. J.; Wemmer, D. E.; Yao, S. Q.; Tian, F.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10654.

(4) (a) Spence, M. M.; Ruiz, E. J.; Rubin, S. M.; Lowery, T. J.; Winssinger, N.; Schultz, P. G.; Wemmer, D. E.; Pines, A. *J. Am. Chem. Soc.* **2004**, *126*, 15287. (b) Chambers, J. M.; Hill, P. A.; Aaron, J. A.; Han, Z. H.; Christianson, D. W.; Kuzma, N. N.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2009**, *131*, 563. (c) Wei, Q.; Seward, G. K.; Hill, P. A.; Patton, B.; Dimitrov, I. E.; Kuzma, N. N.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2006**, *128*, 13274.

(5) Jameson, C. J.; Ruiz, E. J.; Sears, D. N.; Pines, A. *J. Am. Chem. Soc.* **2006**, *128*, 16980.

10.1021/ol300943w

Scheme 1. (A) Synthesis of Trisubstituted Diastereomers of Cryptophane-A from Trihydroxy Cryptophane **1**. (B) Aromatic Region of ^{1}H NMR Spectra of Mixture of Diastereomers, **2a** and **2b**



Diastereomers **2a** and **2b** were isolated by column chromatography with diastereomeric excess ≥98%.

cryptophanes that offer well resolved "bound" and "free" ^{129}Xe NMR peaks should offer substantial advantages. Enantiopure cryptophanes have also been employed for chiral recognition of small guests.[6] Here, we report a new method for producing enantiopure cryptophanes for many different applications.

Until now, the resolution of chiral cryptophanes and hemicryptophanes has typically required expensive HPLC methods and yielded only small quantities of optically pure material.[7] Another approach has been the synthesis of enantiopure cryptophanes from the optically pure CTG units, but one limitation is possible racemization of CTG during the subsequent synthetic steps.[8] Recently, Dutasta and co-workers employed (−)-camphanic chloride as a chiral resolving agent to resolve monocryptophanol through separation of the resulting diastereomers.[9] The diastereomers were not separable by chromatography on silica gel or reversed-phase HPLC, but crystallographic resolution has recently been improved to give both enantiomers in 25% yield.[10] However, this crystallographic method is time-consuming. The low yield of

---

(6) (a) Canceill, J.; Lacombe, L.; Collet, A. *J. Am. Chem. Soc.* **1985**, *107*, 6993. (b) Costante-Crassous, J.; Marrone, T. J.; Briggs, J. M.; McCammon, J. A.; Collet, A. *J. Am. Chem. Soc.* **1997**, *119*, 3818. (c) Crassous, J.; Hediger, S. *J. Phys. Chem. A* **2003**, *107*, 10233. (d) Bouchet, A.; Brotin, T.; Linares, M.; Ågren, H.; Cavagnat, D.; Buffeteau, T. *J. Org. Chem.* **2011**, *76*, 4178. (e) Bouchet, A.; Brotin, T.; Linares, M.; Cavagnat, D.; Buffeteau, T. *J. Org. Chem.* **2011**, *76*, 7816.

(7) (a) Tambute, A.; Canceill, J.; Collet, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1390. (b) Perraud, O.; Raytchev, P. D.; Martinez, A.; Dutasta, J. P. *Chirality* **2010**, *22*, 885.

(8) (a) Canceill, J.; Collet, A.; Gabard, J.; Gottarelli, G.; Spada, G. P. *J. Am. Chem. Soc.* **1985**, *107*, 1299. (b) Garcia, C.; Collet, A. *Bulletin Soc. Chim. Fr.* **1995**, *132*, 52.

(9) Brotin, T.; Barbe, R.; Darzac, M.; Dutasta, J. P. *Chem.—Eur. J.* **2003**, *9*, 5784.

(10) Buffeteau, T.; Cavagnat, D.; Brotin, T. *J. Org. Chem.* **2008**, *73*, 66.

pure cryptophane diastereomers limits the production of enantiomerically pure cages for uses in xenon biosensors and host−guest chemistry, broadly defined.

Dutasta *et al.* previously demonstrated the chromatographic separation of trifunctionalized hemicryptophanes.[11] We hypothesized that a pair of cryptophane-A diastereomers substituted with three chiral auxiliaries would also result in a significant difference in polarity. Indeed, substitution with three chiral resolving groups allowed efficient separation and isolation of cryptophane diastereomers using silica gel column chromatography. Deprotection of the isolated diastereomers yielded the enantiopure trisubstituted cryptophanes, whose chemical and physical properties can be tuned at the three positions.

According to Scheme 1A, diastereomers **2a** and **2b** were synthesized from trihydroxy cryptophane **1**, which was obtained by a previously published six-step route.[12] Trihydroxy cryptophane **1** was reacted with 3.3 equiv of (*S*)-Mosher's acid in the presence of DMAP/Et_3N. The Mosher's acid moiety was chosen as a readily available and sterically bulky chiral resolving agent. The reaction proceeded relatively slowly and went to ~70% completion after stirring for 2 days at 70 °C in DMF. The resulting cryptophane-A diastereomers **2a** and **2b** were successfully separated by column chromatography (silica gel, Et_2O/CH_2Cl_2, 0.5:99.5, v/v) to give each enantiomer in 35% yield. Resolved diastereomers **2a**-(*S*)-(−) and **2b**-(*S*)-(+) were easily distinguished by ^1H NMR spectroscopy (Scheme 1B), each showing four singlets with different chemical shift values for aromatic protons. In contrast, the aromatic region of the diastereomeric mixture exhibited eight singlets in the same region (Scheme 1B). The enantiopurity of the isolated diastereomers was confirmed by electronic circular dichroism (ECD) spectroscopy showing the same peaks with opposite signs (Figure 1a).

The interaction between xenon and the trisubstituted cryptophane diastereomers **2a** and **2b** was investigated by hp ^{129}Xe NMR spectroscopy in a nonintercalating organic solvent, 1,1,2,2-tetrachloroethane-d_2 ($C_2D_2Cl_4$). Hyperpolarized ^{129}Xe was mixed with a sample solution in an airtight NMR tube, and spectra were taken quickly with four transients (Figure 1b). Standarized by the signal from dissolved hp ^{129}Xe in $C_2D_2Cl_4$,[13] hp ^{129}Xe NMR chemical shifts for the mixture of diastereomers **2a**-(−) (67.5 ppm) and **2b**-(+) (77.0 ppm) in $C_2D_2Cl_4$ at 299 K were recorded 9.5 ppm apart (Figure 1b), which is the largest chemical shift difference reported for cryptophane diastereomers. Peaks were assigned by collecting the hp ^{129}Xe NMR spectrum for both of the individual diastereomers (Figure S2). Previously, for the mono-(−)-camphanic acid cryptophane diastereomers, a chemical shift difference of ~7 ppm was observed for the two diastereomers.[14] Notably, for the camphanic acid derivative, the more downfield peak arose from the cryptophane-(−) diastereomer, whereas with three Mosher acids it was the cryptophane-(+) diastereomer. With a 1:1 mixture of diastereomers **2a** and **2b**, the two resonances are clearly resolvable (Figure 1b) by hp ^{129}Xe NMR spectroscopy.

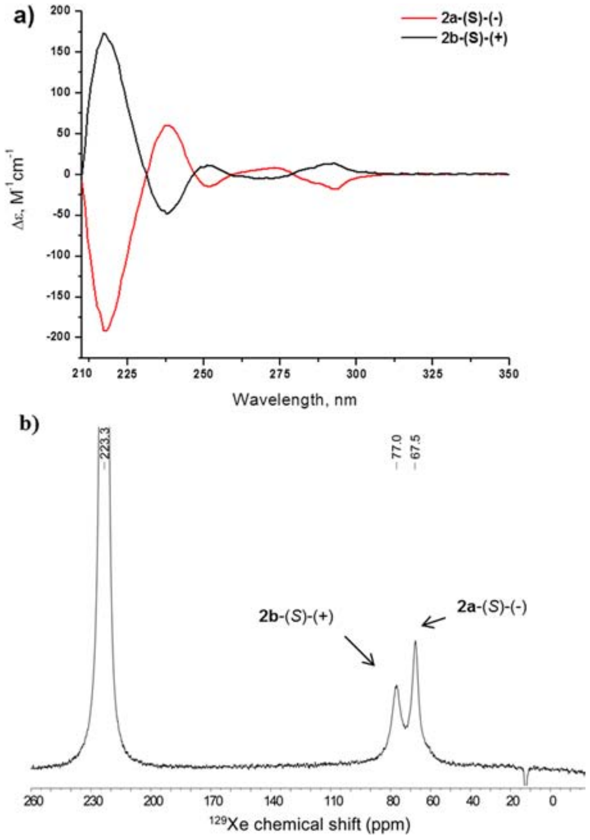


Figure 1. (a) ECD spectra of diastereomers **2a** and **2b** (~0.5 mM) in 1,4-dioxane. (b) Hyperpolarized ^{129}Xe NMR spectra of diastereomers **2a** and **2b** (~10 mM) in $C_2D_2Cl_4$ at 299 ± 2 K.

The isolated cryptophane diastereomers are useful precursors for preparing various enantiopure functionalized cryptophanes. Removal of the Mosher moieties occurs without loss of optical activity. Diastereomers **2a** and **2b** were deprotected via basic hydrolysis at 70 °C, affording enantiopure trihydroxy cryptophanes **3a**-(−) and **3b**-(+) (Scheme 2). The recorded ECD spectra were mirror images (within experimental error) of each other, as expected for a pair of enantiomers (Figure S1a). In the absence of an X-ray crystal structure for the isolated enantiomers, the structural assignment for the two enantiomers was made by reacting cryptophane **3b**-(+) with methyl iodide to yield (+)-cryptophane-A. Its recorded ECD spectrum (Figure S1c) was found to be opposite of the previously reported spectrum for (−)-cryptophane-A.[15]

---

(11) Gautier, A.; Mulatier, J. C.; Crassous, J.; Dutasta, J. P. *Org. Lett.* **2005**, *7*, 1207.

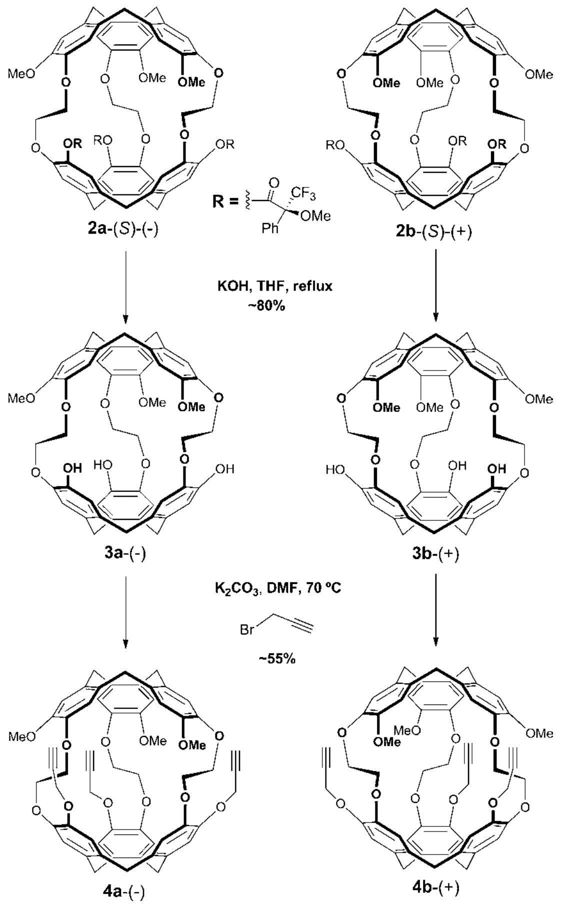
(12) Taratula, O.; Hill, P. A.; Bai, Y. B.; Khan, N. S.; Dmochowski, I. J. *Org. Lett.* **2011**, *13*, 1414.

(13) Bartik, K.; Luhmer, M.; Dutasta, J. P.; Collet, A.; Reisse, J. *J. Am. Chem. Soc.* **1998**, *120*, 784.

(14) Huber, J. G.; Dubois, L.; Desvaux, H.; Dutasta, J. P.; Brotin, T.; Berthault, P. *J. Phys. Chem. A* **2004**, *108*, 9608.

(15) Canceill, J.; Collet, A.; Gottarelli, G.; Palmieri, P. *J. Am. Chem. Soc.* **1987**, *109*, 6454.

Scheme 2. Synthesis of Enantiopure, Trifunctionalized Cryptophanes **3**-(−), **3**-(+), **4**-(−), and **4**-(+)



Similarly to (+)-cryptophane-A, various trisubstituted enantiopure cryptophane derivatives could be easily synthesized from trihydroxy cryptophane enantiomers **3a**-(−) and **3b**-(+). For example, reaction with excess propargyl bromide gave the enantiomerically pure tripropargyl cryptophanes **4a**-(−) and **4b**-(+) (Scheme 2, Figure S1b). We previously showed that alkyl azides can react with tripropargyl cryptophane in nearly quantitative yields via the Cu(I)-catalyzed Huisgen [3 + 2] cycloaddition reaction.[16] This route gave enantiopure tripropargyl cryptophanes **4a**-(−) and **4b**-(+), each in 15% overall yield starting from racemic trihydroxy cryptophane **1**-(±).

In conclusion, an efficient synthesis of enantiopure trifunctionalized cryptophanes was developed using chromatographically resolved trisubstituted cryptophane diastereomers. ECD spectroscopy confirmed the expected chiroptical properties of the isolated diastereomeric and enantiomeric pairs. Hyperpolarized ^{129}Xe NMR chemical shifts were recorded at 9.5 ppm apart for the cryptophane diastereomers. The potential for synthesizing gram-scale quantities of enantiomerically pure cryptophane would provide access to the various functionalized cryptophanes, precursors for many cryptophane-based enantiopure biosensors. Particularly, enantiopure Xe biosensors are desired to facilitate high-resolution X-ray crystallographic studies[1b,4b,17] and to simplify the assignment of peaks in ^{129}Xe NMR spectra.

Acknowledgment. I.J.D. appreciates support from the DOD (W81XWH-04-1-0657), NIH (CA110104 and GM097478), a Camille and Henry Dreyfus Teacher-Scholar Award, and UPenn Chemistry Department. We thank George Furst (Chemistry Department, the University of Pennsylvania) for NMR support.

Supporting Information Available. Experimental procedures and characterization data for all synthesized compounds, ECD and ^{129}Xe NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

---

(16) (a) Tornoe, C. W.; Christensen, C.; Meldal, M. J. *J. Org. Chem.* **2002**, *67*, 3057. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.

(17) (a) Taratula, O.; Hill, P. A.; Khan, N. S.; Carroll, P. J.; Dmochowski, I. J. *Nat. Commun.* **2010**, *1*, 148. (b) Roy, V.; Brotin, T.; Dutasta, J.-P.; Charles, M.-H.; Delair, T.; Mallet, F.; Huber, G.; Desvaux, H.; Boulard, Y.; Berthault, P. *ChemPhysChem* **2007**, *8*, 2082. (c) Kotera, N.; Tassali, N.; Leonce, E.; Boutin, C.; Berthault, P.; Brotin, T.; Dutasta, J.-P.; Delacour, L.; Traore, T.; Buisson, D.-A.; Taran, F.; Coudert, S.; Rousseau, B. *Angew. Chem., Int. Ed.* **2012**, *51*, 4100.