Organic LETTERS

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Host Amplification in a Dithioacetal-Based Dynamic Covalent Library

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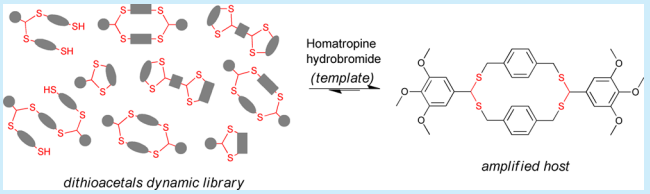
Supporting Information

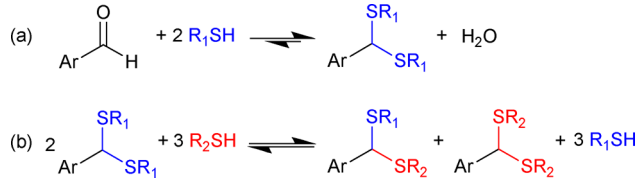
ABSTRACT: Molecular amplification in a dithioacetal-based dynamic library is described for the first time. The homatropine induced selection, amplification, and isolation of one cyclophane host demonstrates the utility of dithioacetal exchange for preparing responsive dynamic libraries. Nuclear magnetic resonance and isothermal titration calorimetry analysis suggest that the amplified macrocycle forms a 1:1 complex with the template. This is the first report about a host/guest system involving a dithioacetal cyclophane.



Dynamic covalent chemistry (DCC)[1] has proven to be a useful tool for the discovery of unexpected receptors,[2] screening of protein inhibitors,[3] and development of new materials.[4] The adaptability toward changes in environment, shown by dynamic libraries, is a distinctive attribute that facilitates the selection of interesting library members for further studies. This library responsiveness is directly affected by the reversible chemistry involved in the preparation of the library. The reaction speed will influence the required time for noticeable changes in composition, and the reaction connectivity will determine the type of products available for amplification.

Recent examples of reversible reactions applied in DCC include orthoester exchange,[5] diselenide exchange,[6] alkyne metathesis,[7] reversible C−C bond formation,[8] reversible native chemical ligation,[9] and peptide sequence exchange.[10] Lately, we have introduced the use of the dithioacetal exchange for the preparation of dynamic libraries. Dithioacetals can be formed and exchanged through fully reversible reactions in the presence of a Brønsted acid in chloroform (Scheme 1).[11] However, there is no example, so far, of molecular amplification from a dithioacetal-based library. In this work we report the use of dithioacetal exchange for the preparation of a dynamic library that responds with the amplification of one macrocyclic host upon introduction of a bioactive alkaloid as a template, and the study of the host/guest complex by NMR and Isothermal Titration Calorimetry (ITC) experiments.

Scheme 1. (a) Formation and (b) Exchange of Dithioacetals



The library design was based on the selection of simple building blocks that can engage in dithioacetal formation and exchange and, by doing so, form molecules that resemble some of the recognition properties of cyclophanes[12] and crown ethers,[13] compound families that have a prominent role in molecular recognition. Dynamic hosts have been generated through olefin metathesis,[14] radical association/dissociation,[15] disulfide exchange,[16] diselenide exchange,[17] acetal exchange,[18] and imine exchange.[19] Such libraries have been the source of cyclophane receptors for alkaloid templates[20] as well as crown ether[21] and mixed cyclophane-crown ether[22] receptors for metal cation templates.

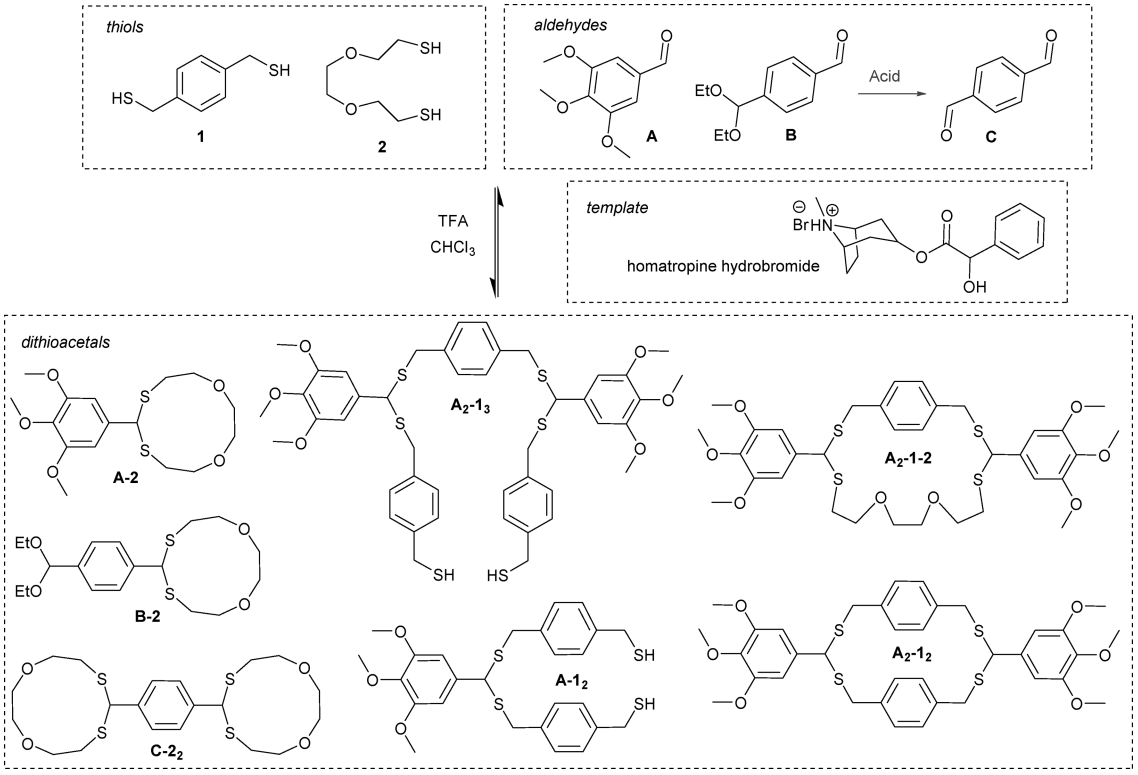
In our case, 3,4,5-trimethoxybenzaldehyde **A** (3 mM) and 4-(diethoxymethyl) benzaldehyde **B** (1.5 mM), together with dithiols 1,4-benzenedimethanethiol **1** (3 mM) and 3,6-dioxa-1,8-octane dithiol **2** (3 mM) (Scheme 2), were dissolved in $CHCl_3$ with TFA (60 mM). After 3 days of reaction, MS analysis showed the presence of a series of library members of unique masses (Table S1). LC-UV-MS analysis showed four main products: cyclic dithioacetals **A-2**, **B-2**, and $\mathbf{A_2}$-$\mathbf{1_2}$, and the linear **A-1**$_2$ (Figure 1a). A significant proportion of starting materials **B**, **C**, and **1** remained unreacted in the equilibrated library.

The dynamic library was exposed to different alkaloids and alkaline metal cations that did not induce any significant composition change; however, when it was prepared in the presence of homatropine hydrobromide (9 mM), a clear shift in the composition was observed. This template is a semisynthetic drug that inhibits the action of acetylcholine in muscarinic receptors,[23] and to the best of our knowledge, there are no

Received: February 8, 2017
Published: March 6, 2017

DOI: 10.1021/acs.orglett.7b00401
Org. Lett. 2017, 19, 1446−1449

Scheme 2. Structure of the Building Blocks, the Template, and the Main Dithioacetal Members Generated in This Study



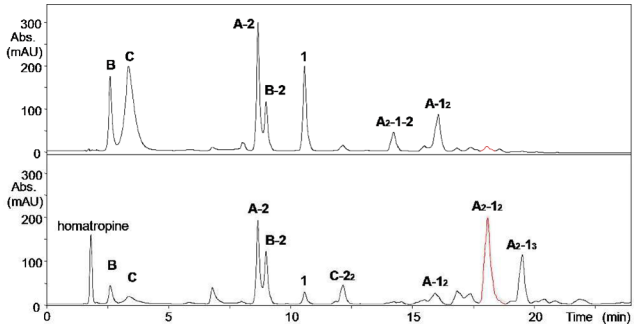


Figure 1. UV-HPLC profile (λ = 250 nm) of the DCL based on dithioacetals (a) in the absence or (b) in the presence of homatropine hydrobromide.

Macrocycle $\mathbf{A_2}$-$\mathbf{1_2}$ was first isolated as a mixture of the *cis* and *trans* isomers as observed by ^{1}H NMR and LC-MS analysis (Figure 2), and then the major isomer was isolated by column

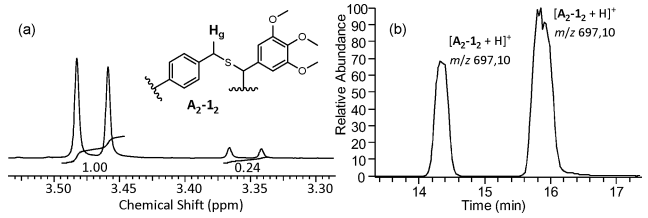


Figure 2. Mixture of isomers of $\mathbf{A_2}$-$\mathbf{1_2}$ in the biased DCL formed from **A** and **1** as evidenced from the (a) partial ^{1}H NMR spectrum and the (b) LC-MS chromatogram monitored for $\mathbf{A_2}$-$\mathbf{1_2}$.

examples of synthetic receptors for homatropine in organic solvents.

The template-induced response of the dynamic library favored mainly the formation of the macrocycle $\mathbf{A_2}$-$\mathbf{1_2}$. This product increases its concentration 18 times in the process (Figure 1b), and it is accompanied by smaller amounts of the linear $\mathbf{A_2}$-$\mathbf{1_3}$ and the bicyclic $\mathbf{C}$-$\mathbf{2_2}$, which also increase their concentration during the templating process.[24]

Macrocycle $\mathbf{A_2}$-$\mathbf{1_2}$ is a dithioacetal cyclophane. Although the first synthesis of a dithioacetal cyclophane is dated more than a century ago[25] literature reports of this type of compound have been scarce[26] and they have usually involved harsh conditions, long reaction times, and low to moderate yields. In order to optimize the amplification of $\mathbf{A_2}$-$\mathbf{1_2}$, a biased library generated from equimolar amounts of building blocks **A** and **1** was prepared in the presence of the template, leading to the formation of $\mathbf{A_2}$-$\mathbf{1_2}$ in 95% yield.

chromatography for further studies.[27] The cyclophane $\mathbf{A_2}$-$\mathbf{1_2}$ has a C_2 symmetry axis, as supported by the symmetric signals observed for each nucleus in the ^{1}H NMR spectrum in $CDCl_3$ (Figure S3a).

Evidence for the supramolecular complexation between $\mathbf{A_2}$-$\mathbf{1_2}$ and homatropine was obtained from ^{1}H NMR experiments in $CDCl_3$ with TFA (15 mM) at 298 K. When homatropine hydrobromide (1.2 mM) was exposed to $\mathbf{A_2}$-$\mathbf{1_2}$ (1.5 mM), shifts of some proton signals from the tropane moiety were observed. The most significant shift was suffered by the NH signal ($\Delta\delta$ = 0.50 ppm), accompanied by smaller shifts of five carbon-bonded proton signals ($\Delta\delta$ < 0.10 ppm) (Figure 3a). When $\mathbf{A_2}$-$\mathbf{1_2}$ (1.5 mM) was exposed to homatropine hydrobromide in excess (30 mM), only minor changes in chemical shift were observed for signals belonging to cyclophane protons. The resonances from the *p*-methoxy groups were slightly changed ($\Delta\delta$ = 0.01 ppm, Figure 3b), whereas no shift could be observed for the aromatic ^{1}H signals.

DOI: 10.1021/acs.orglett.7b00401
Org. Lett. 2017, 19, 1446−1449

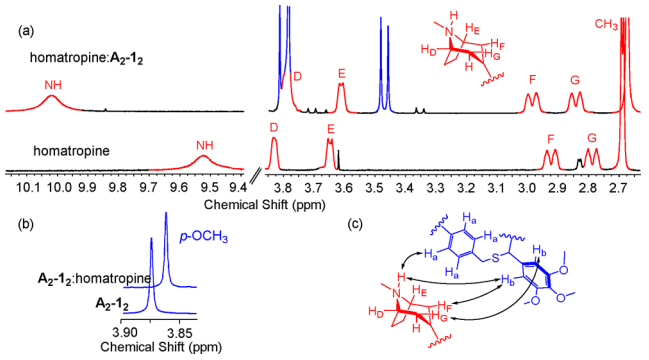


Figure 3. Partial ^{1}H NMR spectra showing the chemical shifts of homatropine (red signals) and $\mathbf{A_2}$-$\mathbf{1_2}$ (blue signals). (a) Homatropine (1.2 mM) in absence and in the presence of $\mathbf{A_2}$-$\mathbf{1_2}$ (blue signals) (1.5 mM) in $CDCl_3$ with TFA (15 mM) or (b) $\mathbf{A_2}$-$\mathbf{1_2}$ (1.5 mM) in absence and in the presence of homatropine (30 mM) in $CDCl_3$ with TFA (15 mM). (c) Observed nOes between $\mathbf{A_2}$-$\mathbf{1_2}$ (90 mM) and homatropine (90 mM) in $CDCl_3$ with TFA (900 mM).

Further evidence of complex formation was obtained from the ^{1}H-NOESY analysis of a 1:1 mixture of $\mathbf{A_2}$-$\mathbf{1_2}$ and homatropine hydrobromide in $CDCl_3$ with TFA. Intermolecular nOes were observed between the NH, H_G, and H_F protons from homatropine and the aromatic H_b proton from the macrocycle (Figures 3c and S4). The NH proton also exhibited an NOE cross peak with the aromatic H_a proton of the cyclophane.

Complex formation was also studied by isothermal titration calorimetry. Since the addition of TFA was necessary in order to ensure complete solubility of the template, the titration conditions used may better resemble the acid conditions present during the selection process in the dynamic library.[28] When $\mathbf{A_2}$-$\mathbf{1_2}$ was titrated into a solution of homatropine hydrobromide, the curve fitting was in agreement with a 1:1 stoichiometry and a binding constant K_{af} of 5.8×10^4 M^{-1} (Figure 4). Formation of the complex was driven by enthalpy (ΔH° = −5.9 kJ mol^{-1}), which exceed the entropic cost

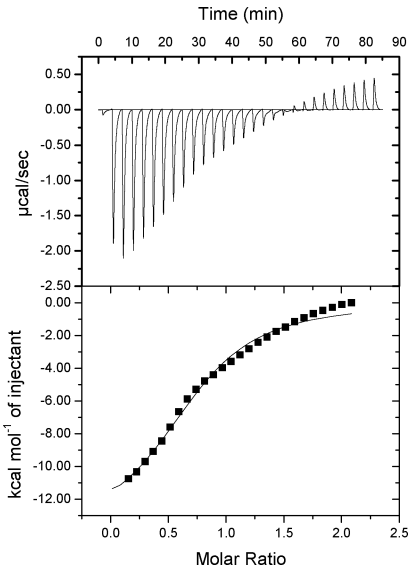


Figure 4. ITC plot obtained upon titrate a chloroform solution of homatropine hydrobromide (2 mM) and TFA (10 mM) into a chloroform solution of $\mathbf{A_2}$-$\mathbf{1_2}$ (0.2 mM).

(−$T\Delta S^\circ$ = 2.9 kJ mol^{-1}) coming from the conformational change of $\mathbf{A_2}$-$\mathbf{1_2}$ or of homatropine upon association. Similar results were obtained when the titration was done at lower or higher concentrations (Figure S6).

In this work, we described the first example of molecular amplification in a dithioacetal-based dynamic library. The selection, amplification, and isolation of one cyclophane host demonstrate the utility of dithioacetal exchange for preparing responsive dynamic libraries. NMR and ITC results suggest that the amplified macrocycle $\mathbf{A_2}$-$\mathbf{1_2}$ forms a 1:1 complex with the template. This is the first report about a host:guest system involving a dithioacetal cyclophane.

Although this study represents a first step in dynamic dithioacetal chemistry, the potential of this reaction in the context of dynamic covalent chemistry and systems chemistry is yet to be exploited.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00401.

Preparation of libraries, identification of the library members by LC-MS, isolation of $\mathbf{A_2}$-$\mathbf{1_2}$, ITC and NMR experiments for the cyclophane:alkaloid complex (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by FONCYT (PICT2015-3574 and PICT2014-3704), CONICET (PIP 695), and Universidad Nacional de Rosario. We thank Andrés Binolfi from the Instituto de Investigaciones para el Descubrimiento de Fármacos de Rosario for his help with binding studies.

■ REFERENCES

(1) (a) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898. (b) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652. (c) Jin, Y.; Yu, C.; Denman, R. J.; Zhang, W. *Chem. Soc. Rev.* **2013**, *42*, 6634.

(2) (a) Cougnon, F. B. L.; Sanders, J. K. M. *Acc. Chem. Res.* **2012**, *45*, 2211. (b) Matache, M.; Bogdan, E.; Hădade, N. D. *Chem. - Eur. J.* **2014**, *20*, 2106. (c) Shyshov, O.; Brachvogel, R.-C.; Bachmann, T.; Srikantharajah, R.; Segets, D.; Hampel, F.; Puchta, R.; von Delius, M. *Angew. Chem.* **2017**, *129*, 794.

(3) (a) Herrmann, A. *Chem. Soc. Rev.* **2014**, *43*, 1899. (b) Mondal, M.; Hirsch, A. K. H. *Chem. Soc. Rev.* **2015**, *44*, 2455. (c) Bandyopadhyay, A.; Gao, J. *Curr. Opin. Chem. Biol.* **2016**, *34*, 110.

(4) (a) Moulin, E.; Cormos, G.; Giuseppone, N. *Chem. Soc. Rev.* **2012**, *41*, 1031. (b) Roy, N.; Bruchmann, B.; Lehn, J.-M. *Chem. Soc. Rev.* **2015**, *44*, 3786. (c) Lehn, J.-M. *Angew. Chem., Int. Ed.* **2015**, *54*, 3276. (d) Zhang, Y.; Barboiu, M. *Chem. Rev.* **2016**, *116*, 809. (e) Jin, Y.; Wang, Q.; Taynton, P.; Zhang, W. *Acc. Chem. Res.* **2014**, *47*, 1575.

DOI: 10.1021/acs.orglett.7b00401
Org. Lett. 2017, 19, 1446−1449

(5) (a) Brachvogel, R.-C.; von Delius, M. *Chem. Sci.* **2015**, *6*, 1399. (b) Brachvogel, R.-C.; von Delius, M. *Eur. J. Org. Chem.* **2016**, *2016*, 3662.
(6) (a) Ji, S.; Cao, W.; Yu, Y.; Xu, H. *Angew. Chem., Int. Ed.* **2014**, *53*, 6781. (b) Rasmussen, B.; Sørensen, A.; Gotfredsen, H.; Pittelkow, M. *Chem. Commun.* **2014**, *50*, 3716.
(7) (a) Lee, S.; Yang, A.; Moneypenny, T. P.; Moore, J. S. *J. Am. Chem. Soc.* **2016**, *138*, 2182. (b) Wang, Q.; Yu, C.; Long, H.; Du, Y.; Jin, Y.; Zhang, W. *Angew. Chem., Int. Ed.* **2015**, *54*, 7550. (c) Wang, Q.; Zhang, C.; Noll, B. C.; Long, H.; Jin, Y.; Zhang, W. *Angew. Chem., Int. Ed.* **2014**, *53*, 10663.
(8) Beaudoin, D.; Levasseur-Grenon, O.; Maris, T.; Wuest, J. D. *Angew. Chem., Int. Ed.* **2016**, *55*, 894.
(9) Ruff, Y.; Garavini, V.; Giuseppone, N. *J. Am. Chem. Soc.* **2014**, *136*, 6333.
(10) Pappas, C. G.; Shafi, R.; Sasselli, I. R.; Siccardi, H.; Wang, T.; Narang, V.; Abzalimov, R.; Wijerathne, N.; Ulijn, R. V. *Nat. Nanotechnol.* **2016**, *11*, 960.
(11) Orrillo, A. G.; Escalante, A. M.; Furlan, R. L. E. *Chem. - Eur. J.* **2016**, *22*, 6746.
(12) *Modern cyclophane chemistry*, Gleiter, R., Hopf, H., Eds.; Wiley-VCH: Weinheim, 2004.
(13) (a) Christensen, J. J.; Hill, J. O.; Izatt, R. M. *Science* **1971**, *174*, 459. (b) Gokel, G. W.; Leevy, W. M.; Weber, M. E. *Chem. Rev.* **2004**, *104*, 2723.
(14) (a) Monfette, S.; Fogg, D. E. *Chem. Rev.* **2009**, *109*, 3783.
(15) Kobashi, T.; Sakamaki, D.; Seki, S. *Angew. Chem., Int. Ed.* **2016**, *55*, 8634.
(16) (a) Brisig, B.; Sanders, J. K. M.; Otto, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1270. (b) Corbett, P. T.; Sanders, J. K. M.; Otto, S. *J. Am. Chem. Soc.* **2005**, *127*, 9390. (c) Corbett, P. T.; Tong, L. H.; Sanders, J. K. M.; Otto, S. *J. Am. Chem. Soc.* **2005**, *127*, 8902. (d) Vial, L.; Ludlow, R. F.; Leclaire, J.; Pérez-Fernández, R.; Otto, S. *J. Am. Chem. Soc.* **2006**, *128*, 10253. (e) Hamieh, S.; Saggiomo, V.; Nowak, P.; Mattia, E.; Ludlow, R. F.; Otto, S. *Angew. Chem., Int. Ed.* **2013**, *52*, 12368. (f) James, L. I.; Beaver, J. E.; Rice, N. W.; Waters, M. L. *J. Am. Chem. Soc.* **2013**, *135*, 6450. (g) Fanlo-Virgós, H.; Alba, A.-N. R.; Hamieh, S.; Colomb-Delsuc, M.; Otto, S. *Angew. Chem., Int. Ed.* **2014**, *53*, 11346. (h) Skowron, P.-T.; Dumartin, M.; Jeamet, E.; Perret, F.; Gourlaouen, C.; Baudouin, A.; Fenet, B.; Naubron, J.-V.; Fotiadu, F.; Vial, L.; Leclaire, J. *J. Org. Chem.* **2016**, *81*, 654. (i) Collins, M. S.; Carnes, M. E.; Nell, B. P.; Zakharov, L. N.; Johnson, D. W. *Nat. Commun.* **2016**, *7*, 11052.
(17) Thomas, J.; Dobrzańska, L.; Van Meervelt, L.; Quevedo, M. A.; Woźniak, K.; Stachowicz, M.; Smet, M.; Maes, W.; Dehaen, W. *Chem. - Eur. J.* **2016**, *22*, 979.
(18) (a) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *J. Am. Chem. Soc.* **2005**, *127*, 13666. (b) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *Chem. - Eur. J.* **2006**, *12*, 8566. (c) Cacciapaglia, R.; Di Stefano, S.; Mandolini, L.; Mencarelli, P.; Ugozzoli, F. *Eur. J. Org. Chem.* **2008**, *2008*, 186. (d) Cacciapaglia, R.; Di Stefano, S.; Ercolani, G.; Mandolini, L. *Macromolecules* **2009**, *42*, 4077. (e) Berrocal, J. A.; Cacciapaglia, R.; Di Stefano, S.; Mandolini, L. *New J. Chem.* **2012**, *36*, 40. (f) Ruggi, A.; Cacciapaglia, R.; Di Stefano, S.; Bodo, E.; Ugozzoli, F. *Tetrahedron* **2013**, *69*, 2767.
(19) (a) Glink, P. T.; Oliva, A. I.; Stoddart, J. F.; White, A. J. P.; Williams, D. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 1870. (b) Aricó, F.; Chang, T.; Cantrill, S. J.; Khan, S. I.; Stoddart, J. F. *Chem. - Eur. J.* **2005**, *11*, 4655. (c) Leung, K. C.-F.; Aricó, F.; Cantrill, S. J.; Stoddart, J. F. *J. Am. Chem. Soc.* **2005**, *127*, 5808. (d) Ulrich, S.; Buhler, E.; Lehn, J.-M. *New J. Chem.* **2009**, *33*, 271. (e) Mondal, B.; Acharyya, K.; Howlader, P.; Mukherjee, P. S. *J. Am. Chem. Soc.* **2016**, *138*, 1709. (f) McCaffrey, R.; Long, H.; Jin, Y.; Sanders, A.; Park, W.; Zhang, W. *J. Am. Chem. Soc.* **2014**, *136*, 1782.
(20) (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590. (b) Hamieh, S.; Ludlow, R. F.; Perraud, O.; West, K. R.; Mattia, E.; Otto, S. *Org. Lett.* **2012**, *14*, 5404.
(21) Marsella, M. J.; Maynard, H. D.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1101.
(22) Houjou, H.; Iwasaki, A.; Ogihara, T.; Kanesato, M.; Akabori, S.; Hiratani, K. *New J. Chem.* **2003**, *27*, 886.
(23) Weiner, N. In *The Pharmacological Basis of Therapeutics*, 7th ed.; Goodman, L. S., Gilman, A., Rall, T. W., Murad, F., Eds.; Macmillan: New York, 1985.
(24) Although the amplification of linear species from dynamic libraries is not very common, amplification of $\mathbf{A}_2$-$\mathbf{1}_3$ was observed in libraries prepared from **A** + **B** + **1** + **2** under several conditions. For an example, see Figure S8.
(25) Autenrieth, W.; Beuttel, F. *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 4346.
(26) (a) Barber, H. J.; Smiles, S. *J. Chem. Soc.* **1928**, *0*, 1141. (b) Pedersen, C. J. *J. Org. Chem.* **1971**, *36*, 254. (c) Allen, D. W.; Braunton, P. N.; Millar, I. T.; Tebby, J. C. *J. Chem. Soc. C* **1971**, 3454. (d) Vögtle, F.; Lichtenthaler, R. *Z. Naturforsch.* **1971**, *26b*, 872. (e) Vögtle, F.; Lichtenthaler, R. G. *Tetrahedron Lett.* **1972**, *13*, 1905. (f) Vögtle, F.; Lichtenthaler, R. G. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 535. (g) Busby, R. E.; Huckle, D. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1705. (h) Bottino, F.; Pappalardo, S. *Tetrahedron* **1980**, *36*, 3095. (i) Bottino, F.; Pappalardo, S. *Chem. Lett.* **1981**, *10*, 1781. (j) Takemura, T.; Kozawa, K.; Uchida, T.; Mori, N. *Chem. Lett.* **1984**, *13*, 1839. (k) Beer, P. D.; Drew, M. G. D.; Ibbotson, A.; Lacy, S. M. *Tetrahedron* **1997**, *53*, 3155.
(27) Only the major isomer was isolated to carry out complexation studies. Isolation of the minor isomer was elusive because of the very similar chromatography behavior of both isomers.
(28) (a) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210. (b) Chung, M.-K.; Lee, S. J.; Waters, M. L.; Gagné, M. R. *Chem. Commun.* **2016**, *52*, 8103.

DOI: 10.1021/acs.orglett.7b00401
Org. Lett. 2017, 19, 1446−1449