Tetrahedron Letters 57 (2016) 2539–2543

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Azobenzene-bridged bile acid dimers: an interesting class of conjugates with conformation-controlled bioactivity

Weina Li [a,†], Yan Li [b,†], Xianpeng Yin [b], Yun Liang [b], Jian Li [b], Chen Wang [b], Yue Lan [b], Hui Wang [b], Yong Ju [b,*], Guangtao Li [b,*]

[a] College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China
[b] Department of Chemistry, Tsinghua University, Beijing 10084, China

ARTICLE INFO

Article history:
Received 4 March 2016
Revised 26 April 2016
Accepted 29 April 2016
Available online 30 April 2016

Keywords:
Conformation
Antibacterial activity
Bile acid
Azobenzene
Dimers

ABSTRACT

The synergetic combination of the distinct properties of azobenzene and bile acid could afford stable tweezer-like conformation with tunable hydrophilic and hydrophobic channels, thus increasing their antimicrobial activity toward both Gram-positive and Gram-negative bacteria, which can be conveniently switched off when the conformation turn back to the extended state.

In nature, the essential functions of the majority of biomolecules, such as the binding properties of protein receptors and the catalytic activity of enzymes, are regulated through their highly sophisticated conformation changes.[1] The interconnectivity between conformation and bioactivity could also provide outstanding potential for studying organism development or disease progression.[2] However, in chemical systems controlling and manipulating the conformation and activity of man-made materials in a reversible manner is very difficult[3] and only several limited examples were presented.[4]

Recently, it is revealed that synthetic molecules with controlled bioactivity 'on' and 'off' by conformational change have great advantage when used as antibacterial agents.[5] The selective activation/inactivation is useful for reducing the accumulation of drugs in the environment and will pave the way to address the challenges associated with the emergence of drug resistance.[6] Azobenzene with reversible light-induced isomerization has been well-demonstrated to be suited for induction of local conformational changes into various molecules, such as peptides, proteins and nucleic acids, and thus leading to the regulation of corresponding bioactivity.[7] In this respect, some outstanding examples with this conformation switchable groups modified the well-known antibacterial active compounds have been presented.[8] However, developing new activity-switchable drugs without the traditional active groups has long been attractive and is helpful for circumventing the problem of drug resistance.

Bile acids are naturally occurring amphiphilic compounds. Unlike the traditional 'head–tail' amphiphilic molecules, they have a curved steroidal skeleton with polar hydroxyl groups on one face and nonpolar hydrophobic methyl groups on the other face, thus they exhibit the facial amphiphilicity.[9] Although they are not traditional active antimicrobial drugs, due to the unique features the derivatives of bile acids are still pharmacologically interesting and some bile acid dimers and oligomers have been demonstrated with antiproliferative and antifungal activities.[10] It is revealed that these cholic acid derived facial amphiphiles with cleft or umbrella conformation can improve the permeability or destroy the integrity of membranes such as bacterial cell walls, thus leading to the impressive antimicrobial activities.[11] This kind of antibiotics without the traditional active compounds has great advantages to address the emergence of drug resistance. However, the effectiveness of the above mentioned bile acid derivatives are highly dependent on their polar solvent-induced conformation, which are not stable and predictable.

With the aim of developing new stable and predictable conformation-controlled antibacterial agents without using the traditional active antimicrobial compounds, in this work we synthesized a series of azobenzene-bridged bile acid conjugates

* Corresponding authors. Tel.: +86 1062792905.
E-mail address: lgt@mail.tsinghua.edu.cn (G. Li).
† These authors contributed equally to this work.

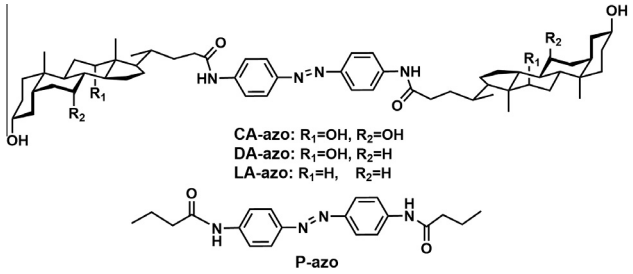
http://dx.doi.org/10.1016/j.tetlet.2016.04.107
0040-4039/

and explored their conformation-bioactivity interconnectivity. It was found that the synergetic combination of the distinct properties of azobenzene and bile acid moieties, the stable and controllable tweezer conformation with tunable hydrophilic and hydrophobic channels can be obtained conveniently. Interestingly, at this tweezer-like conformation the antibacterial activity of the synthesized bile acid conjugates for both Gram-positive and Gram-negative bacteria increased apparently. However, when the molecules conformation changed back to the extended state, the antibacterial activity decreased simultaneously. For azobenzene-bridged deoxycholic acid dimers, the antibacterial activity was even switched off for Gram-positive bacteria. More importantly, this bioactivity conversion can be carried out reversibly upon the molecule conformation changes from the extended state to the tweezer-like form.

Scheme 1 shows the chemical structures of synthesized azobenzene linked dimeric cholic acid (**CA-azo**), deoxycholic acid (**DA-azo**), and lithocholic acid (**LA-azo**). Attempts to directly condense the bile acid with 4,4′-azodianiline in the presence of DCC proved to be rather sluggish and afforded a complex mixture of products. In an alternative way (Scheme S1), the hydroxyl groups of bile acid were first protected by formylation with anhydrous formic acid in almost quantitative yield. Then coupling of the acid chloride with diaminoazobenzene in the presence of triethylamine gave the bis-coupled products. Hydrolysis of the diamide with LiOH in THF–H_2O led to the desired bile acid dimers in about 80% yield. The three kinds of molecules are differentiated only by the number of hydroxyl groups. As the model compound for comparison, azobenzene with two butane end groups (**P-azo**) was also synthesized.

It was well recognized that azobenzene can undergo photoisomerization from a full-conjugated *trans* configuration to a *cis* isomer under illumination by 365 nm light.[12] Thermal *cis* to *trans* relaxation in the dark leads to 100% *trans* isomer reversibly. As demonstrated by ^{1}H NMR spectra of azobenzene-bridged bile acid dimers (Figs. S15, S17, S19), the peaks at about 7.80 ppm are ascribed to the aromatic protons of *trans* isomers and no peaks belonging to the *cis* isomers were observed,[13] revealing a pure *trans* isomers before irradiating by 365 nm light. The extended *trans* conformation can change to a tweezer-like state in a reversible manner. And the tweezer-like conformation is expected to be adaptable to the polarity of the surroundings and led to the tunable hydrophilic and hydrophobic channels (Fig. 1a). Due to the facial amphiphilic feature of the pendant bile acid moieties, the tweezer-like conformation can be more predictable and stable, thereby facilitating our effort to evaluate the bioactivity of these two different conformation states and further probe the conformation-bioactivity relationship of these bile acid derivatives.

The conformation switchable behavior of these azobenzene-bridged bile acid dimers and the model compound **P-azo** was first investigated. When irradiating the **CA-azo** solution (1 mM in CH_3-OH) with 365 nm light, the absorption band at 371 nm which is attributed to the π–π^* transition of the *trans*-azobenzene decreases, along with an increase of the band around 455 nm, which is attributed to the n–π^* absorption (Fig. 1b). This result indicates that the **CA-azo** undergoes the expected conformation change and the Uv–vis spectra further confirmed the presence of pure *trans* isomers before irradiation by 365 nm light. The isomerization of **CA-azo** proceeds relatively slowly compared to the model compound **P-azo** (Fig. 1c), which only need less than 3 min to complete isomerization. This phenomenon can be easily understood by the fact that the azo unit of **CA-azo** was placed at core of the molecule which was surrounded by two large and rigid steroid skeletons, so that its conformation state change encounters more significant resistance than **P-azo** with only two short and flexible end groups.



Scheme 1. Chemical structure of the azobenzene linked bile acid dimers (**CA-azo**, **DA-azo** and **LA-azo**) and the model compound (**P-azo**).

However, the further investigation proves that the rates of the conformation change of these three bile acid-azobenzene conjugates are also varied and strongly affected by the subtle variation of the pendant groups, especially in the relative concentrated solution (~10 mg/mL). For this concentration, ^{1}H NMR measurement can be performed to calculate the ratio of different configurations accurately by the integral of the corresponding signals. As shown in Figure S1, for **CA-azo** the fraction of the tweezer-like conformation is much lower than that of **LA-azo** under the same intensity of light irritation. The conformation change rate of **DA-azo**, which possesses two hydroxyl groups at the steroid nucleus, lies right in between the values of **CA-azo** and **LA-azo**. It is clear that the only thing different between the three bile acid derivatives is the number of the hydroxyl groups on the skeleton, so the different rate of isomerization could no longer be simply explained by the steric hindrance due to size of the pendant groups. In the concentrated solution (Scheme S2), it was assumed that the hydrophobic face of bile acid tends to aggregate together in the polar media, leaving the hydrophilic groups pointing toward the solvents and reducing the interfacial energy. The hydroxyl groups are expected to form hydrogen bonds with the solvent molecules. The more hydroxyl groups the compounds present, the stronger hydrogen bond will be formed between the pendant groups and solvent molecules. Therefore, not only the size of the pendant group, but also the properties of the substituents around the azobenzene core determine the rate and activation energy barrier for the conformation change process.

Bridged by azobenzene groups, these bile acid derivatives with tweezer-like configuration tend to revert to the extended state thermally once the optical irritation was absence. This behavior may interfere with our bioactivity test and leave unpredictable result for the tweezer-like configuration, so it is necessary to characterize the stability of the tweezer-like state before the antimicrobial screen. In order to obtain a useful reference for the conversion rate during the bioactivity test, the tweezer-like state of both **CA-azo** and **P-azo** in solution was placed in dark at 30 °C, which is the temperature that the bacteria were cultured. Then the kinetics of the thermal-induced conformation reversion was measured by following the changes in absorbance at 371 nm. As displayed in Figure S2, the thermal half-lives ($\tau_{1/2}$) of **CA-azo** are about 3.5 h, which was found to be extraordinarily extended as compared to **P-azo** ($\tau_{1/2}$ < 1.5 h) in CH_3OH. The result indicated that once the molecule conformation changed to tweezer-like state, **CA-azo** presented a higher thermal-energy barrier to convert back to the extended form compared with that of **P-azo**. Besides the above mentioned steric hindrance effect, this phenomenon could also be contributed to the formation of tightly folded conformation by hydrophobic and hydrophilic interactions in solvents. In other words, the tweezer-like configuration of bile acid dimers is more stable than other typical azo derivatives.

As demonstrated by the conformation change of bile acid derivatives, the 100% tweezer-like isomers is not expected to be

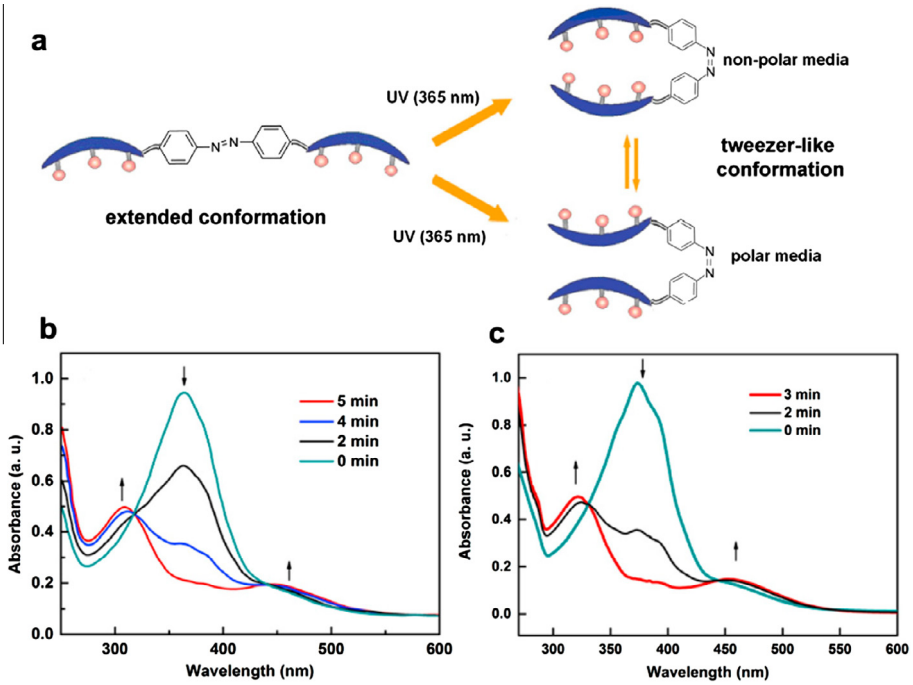


Figure 1. (a) Schematic representation of conformation change of azobenzene linked cholic acid derivatives: from extended state to tweezer-like conformation; UV–vis spectra of conformation change procedure of (b) **CA-azo** and (c) **P-azo** under the irradiation at 365 nm UV light; spectra are normalized to the maximum π–π^* absorption around 371 nm.

maintained during the antibacterial test. However, the slow thermal re-isomerization allows us to evaluate the antibacterial activity of these two configurations respectively. Two bacterial cultures, such as *Escherichia coli* (Gram-negative bacteria) and *Bacillus megaterium* (Gram-positive bacteria) were selected. For *E. Coli*, only modest (ca. 40% bacteriostasis rate) antibacterial property was observed for **CA-azo** and **DA-azo** in the extended conformation (Figs. 2a and b, S3). When the compounds were exposed to 365 nm light for 15 min, the tweezer-like conformation was formed and a considerable increase of the bacteriostasis rate was observed. More than 80% bacterial growth was inhibited at 100 μg/mL concentration of these two bile acid derivatives (Fig. S3), which suggests that the tweezer-like conformation exhibits stronger antibacterial activity compared to the corresponding extended structure.

Compared to *E. coli*, these bile acid derivatives show relative weak inhibitory effect toward Gram-positive bacteria *Bacillus megaterium*, especially for **DA-azo**. As demonstrated in Figure 2d, the extended configuration of **DA-azo** exhibits almost no antibacterial activity even in concentrations up to 100 μg/mL. It is also demonstrated that the difference of bacteria inhibitory effect between **CA-azo** and **DA-azo** toward the Gram-positive bacteria becomes more pronounced, about 20% bacteriostasis rate discrepancies, which is much greater than that toward Gram-negative bacteria (Fig. 2c, d). However, the similar phenomenon to the Gram-negative bacteria is that the obvious increase of the bacteriostasis rate was also observed when the conformation transferred to tweezer-like state. Due to the limited solubility in aqueous solution, the antibacterial activity of compound **LA-azo** and **P-azo** was not tested.

To further examine the switchable biological properties, we evaluate the antibacterial activity of **CA-azo** under alternating irradiation at 365 and 450 nm light, thus the conformation of **CA-azo** changes from extended form to a stable tweezer-like conformation (Fig. 3). When the molecular conformation is turning to stable tweezer-like state gradually, the bacteriostasis rate undergoes a steady increase and finally up to around 80%, which is consistent with the antibacterial test results in Figure 2. The recovery of the inhibitory effect to the initial state was observed when the sample was converted to the extended state. The nearly periodical change of the antibacterial activity of azobenzene-bridged bile acid dimers is a clear evidence of the interconnectivity of the conformation and bioactivity. The conformation-controlled antibacterial activity can be useful to address the accumulation of drugs in the environment and decrease the drug resistance.

According to the literature reports, ion channel mechanism plays a very important role for many bile acid derived antibacterial agents,[14] in which the facially amphiphilic structures form a hydrophilic pore and produce ionic fluxes across biological membranes. In order for ions or molecules to be transported across the hydrophobic membrane barriers, the bile acid derivatives must be to some extent insert into the double layer phospholipids. In this respect, the tweezer-like configuration of azobenzene-bridged bile acid dimers has the great advantages to facilitate the formation of hydrophilic channels, thereby avoiding the unfavorable interaction between the hydrophobic lipids and the hydrophilic face of the bile acid moieties (Scheme 2). After the insertion of the bile acid dimers into phospholipids, it promotes the mass transport across the bacterial outer membrane, which accounts for the strong antimicrobial activity. And the better performance toward other inhibitors may also be ascribed to this special interaction mechanism. The extended conformation of azobenzene-bridged bile acid dimers may produce intermolecular hydrogen bonds in non-polar media and insert into the membrane, as it was presented by Kobuke et al.[15] However, our experiment demonstrates that the tweezer-like configuration could form the ion channels more efficiently due to the unique preorganization of the amphiphilic structure.

The reason for lower efficiency of these bile acid dimers in bacteriostasis rate toward Gram-positive compared with Gram-negative bacteria is not very clear, but similar phenomena have been observed for some antimicrobial peptides.[16] Unlike Gram-negative

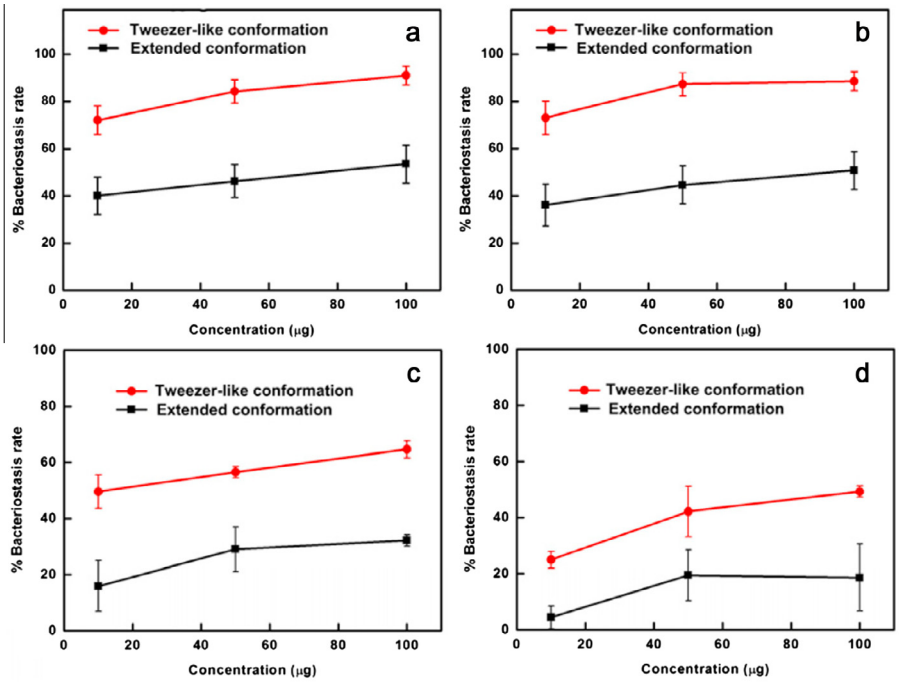


Figure 2. Graphical representation of in vitro antibacterial tests toward *Escherichia coli 5α* (a, b) and *Bacillus megaterium* (c, d) at tweezer-like and extended conformation: (a, c) **CA-azo**, (b, d) **DA-azo**.

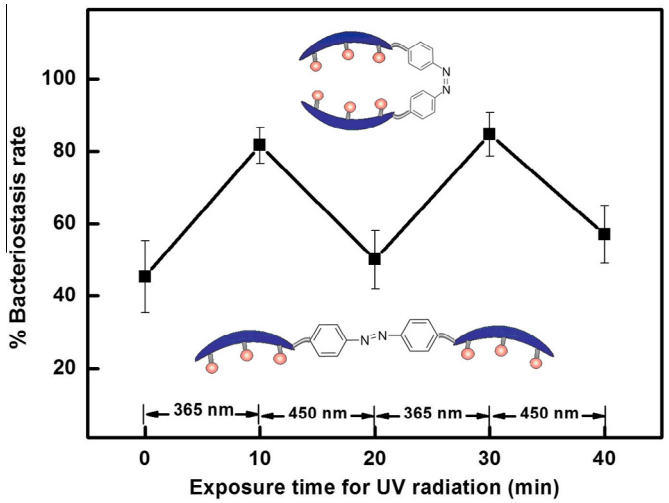
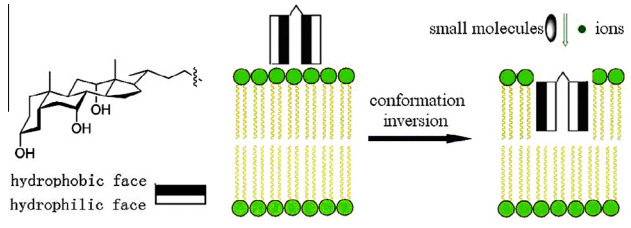


Figure 3. Conformation-regulated antibacterial property of **CA-azo** against *E. coli* in the concentration of 50 μg/mL.

bacteria, the outer layer of Gram-positive bacteria is thick and rigid, consisting of a cross-linked peptidoglycan.[17] Perhaps the thick and cross-linked peptidoglycan layer greatly hinders the permeation of the bile acid dimers, thereby protecting the membrane of the Gram-positive from disruption.



Scheme 2. Proposed mechanism for the azobenzene-bridged bile acid dimers being drawn into a lipid bilayer in their tweezer-like configuration. Here, the azobenzene bridge has been omitted for simplicity.

In summary, a new series of conformation-controlled antibacterial agents based on bile acid-azobenzene conjugates have been developed. By combination the distinct properties of azobenzene and bile acid units, these amphiphilic conjugates undergo remarkable conformation changes from the extended form to tweezer-like conformation in a predictable and reversible manner. Interestingly, upon the conformation changing to tweezer-like form, the antimicrobial activity of the synthesized derivatives for both Gram-positive and Gram-negative bacteria was increased, which can be switched back conveniently by the conformation regulation. This work demonstrates an interesting class of conformation-controlled antibacterial agents based on the unconventional antimicrobial activity groups, which may be useful for circumventing the problem of drug resistance.

Acknowledgments

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (21025311, 21121004, 91027016, and 21403122), MOST Programs (2011CB808403 and 2013CB834502) and the Deutsche Forschungsgemeinscha DFG (TRR61).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.04.107.

References and notes

1. (a) Krauss, U.; Drepper, T.; Jaeger, K. E. *Chem. Eur. J.* **2011**, *17*, 2552; (b) Sutherland, S. *Nature* **1985**, *315*, 258.
2. (a) Carreno, M. C.; Garcia, I.; Nunez, I.; Merino, E.; Ribagorda, M.; Pieraccini, S.; Spada, G. P. *J. Am. Chem. Soc.* **2007**, *129*, 7089; (b) Deisseroth, K. *Nat. Methods* **2011**, *8*, 26.

3. Fehrentz, T.; Schoenberger, M.; Trauner, D. *Angew. Chem., Int. Ed.* **2011**, *50*, 12156.
4. (a) Beharry, A. A.; Woolley, G. A. *Chem. Soc. Rev.* **2011**, *40*, 4422; (b) Muraoka, T.; Koh, C. Y.; Cui, H.; Stupp, S. I. *Angew. Chem., Int. Ed.* **2009**, *48*, 5946; (c) Szymanski, W.; Beierle, J. M.; Kistemaker, H. A. V.; Velema, W. A.; Feringa, B. L. *Chem. Rev.* **2013**, *113*, 6114; (d) Binschik, J.; Zettler, J.; Mootz, H. D. *Angew. Chem., Int. Ed.* **2011**, *50*, 3249.
5. Velema, W. A.; van der Berg, J. P.; Hansen, M. J.; Szymanski, W.; Driessen, A. J. M.; Feringa, B. L. *Nat. Chem.* **2013**, *5*, 924.
6. Velema, W. A.; Szymanski, W.; Feringa, B. L. *J. Am. Chem. Soc.* **2014**, *136*, 2178.
7. (a) Rudiuk, S.; Venancio-Marques, A.; Baigl, D. *Angew. Chem., Int. Ed.* **2012**, *51*, 12694; (b) Wysoczanski, P.; Mart, R. J.; Loveridge, E. J.; Williams, C.; Whittaker, S. B. M.; Crump, M. P.; Allemann, R. K. *J. Am. Chem. Soc.* **2012**, *134*, 7644; (c) Zhang, F.; Timm, K. A.; Arndt, K. M.; Woolley, G. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 3943.
8. (a) Goestl, R.; Hecht, S. *Chem. Eur. J.* **2015**, *21*, 4422; (b) Stein, M.; Middendorp, S. I.; Carta, V.; Pejo, E.; Raines, D. E.; Forman, S. A.; Sigel, E.; Trauner, D. *Angew. Chem., Int. Ed.* **2012**, *51*, 10500; (c) Velema, W. A.; van der Berg, J. P.; Szymanski, W.; Driessen, A. J. M.; Feringa, B. L. *ACS Chem. Biol.* **2014**, *9*, 1969.
9. (a) Zhang, M.; Yin, X. P.; Tian, T.; Liang, Y.; Li, W. N.; Lan, Y.; Li, J.; Zhou, M. M.; Ju, Y.; Li, G. T. *Chem. Commun.* **2015**, 10210; (b) Li, W. N.; Li, X. S.; Zhu, W.; Li, C. X.; Xu, D.; Ju, Y.; Li, G. T. *Chem. Commun.* **2011**, 7728; (c) Ivanysenko, O.; Strandman, S.; Zhu, X. X. *Polym. Chem.* **2012**, *3*, 1962.
10. (a) Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1591; (b) Vandenburg, Y. R.; Smith, B. D.; Perez-Payan, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2000**, *122*, 3252; (c) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Deshpande, M. V.; Kadreppa, S.; Chattopadhyay, S.; Gonnade, R. G. *Org. Biomol. Chem.* **2008**, *6*, 3823.
11. (a) Janout, V.; Jing, B. W.; Regen, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 15862; (b) Janout, V.; Zhang, L. H.; Staina, I. V.; Di Giorgio, C.; Regen, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 5401.
12. (a) Natansohn, A.; Rochon, P. *Chem. Rev.* **2002**, *102*, 4139; (b) Nalluri, S. K. M.; Voskuhl, J.; Bultema, J. B.; Boekema, E. J.; Ravoo, B. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 9747; (c) Stoffelen, C.; Voskuhl, J.; Jonkheijm, P.; Huskens, J. *Angew. Chem., Int. Ed.* **2014**, *53*, 3400; (d) Ingale, G.; Seo, Y. J. *Tetrahedron Lett.* **2014**, *55*, 5247.
13. (a) Kang, H. C.; Lee, B. M.; Yoon, J.; Yoon, M. J. *Colloid Interface Sci.* **2000**, *231*, 255; (b) Coelho, P. J.; Castro, M. C. R.; Fernandes, S. S. M.; Fonseca, A. M. C.; Raposo, M. M. M. *Tetrahedron Lett.* **2012**, *53*, 4502.
14. (a) Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 6138; (b) Kukuchi, K.; Bernard, E. M.; Sadownik, A.; Regen, S. L.; Armstrong, D. *Antimicrob. Agents Chemother.* **1997**, *41*, 1433.
15. (a) Kobuke, Y.; Nagatani, T. *J. Org. Chem.* **2001**, *66*, 5094; (b) Goto, C.; Yamamura, M.; Satake, A.; Kobuke, Y. *J. Am. Chem. Soc.* **2001**, *123*, 12152.
16. Zasloff, M. *Nature* **2002**, *415*, 389.
17. Scott, M. G.; Gold, M. R.; Hancock, R. E. W. *Infect. Immun.* **1999**, *67*, 6445.