

### Siderophore Conjugates

International Edition: DOI: 10.1002/anie.201603921 German Edition: DOI: 10.1002/ange.201603921

# Facile and Versatile Chemoenzymatic Synthesis of Enterobactin Analogues and Applications in Bacterial Detection

Albert A. Lee, Yi-Chen S. Chen, Elisa Ekalestari, Sheng-Yang Ho, Nai-Shu Hsu, Tang-Feng Kuo, and Tsung-Shing Andrew Wang\*

Abstract: Siderophores, such as enterobactin (Ent), are small molecules that can be selectively imported into bacteria along with iron by cognate transporters. Siderophore conjugates are thus a promising strategy for delivering functional reagents into bacteria. In this work, we present an easy-to-perform, one-pot chemoenzymatic synthesis of functionalized monoglucosylated enterobactin (MGE). When functionalized MGE is conjugated to a rhodamine fluorophore, which affords RhB-Glc-Ent, it can selectively label Gram-negative bacteria that utilize Ent, including some E. coli strains and P. aeruginosa. V. cholerae, a bacterium that utilizes linearized Ent, can also be weakly targeted. Moreover, the targeting is effective under ironlimiting but not iron-rich conditions. Our results suggest that the RhB-Glc-Ent probe is sensitive not only to the bacterial strain but also to the iron condition in the environment.

Siderophores are secondary metabolites, which are secreted by microorganisms, with high chelation affinity for iron(III).<sup>[1]</sup> These molecules acquire iron(III) from extracellular space under iron-limiting conditions, and are critical to microbe survival.<sup>[2]</sup> Siderophores differ between microbe species and exhibit highly variable chemical structures.<sup>[1c,3]</sup> The ironbound siderophores are recognized and transported into the cells by dedicated receptors.<sup>[4]</sup> Only the microbes expressing the cognate receptor can take up the siderophore.<sup>[5]</sup> Owing to the selective and essential nature of siderophore-based iron acquisition, siderophores can potentially serve as a probe to target microbes.

Natural siderophore mimics, or sideromycins, selectively deliver toxic cargos into bacteria by conjugating toxins.<sup>[6]</sup> These mimics demonstrate the feasibility of the siderophore-based targeting strategy and have inspired the development of many synthetic conjugates, including antibiotic conjugates for Trojan-horse-type delivery,<sup>[7]</sup> and surface-conjugated siderophores for bacterial adsorption and detection.<sup>[8]</sup> All of these applications require a handle on the siderophore for site-specific modification.

[*]	A. A. Lee, YC. S. Chen, SY. Ho, NS. Hsu, TF. Kuo, Dr. TS. A. Wang
	Department of Chemistry, National Taiwan University
	No. 1, Sec. 4, Roosevelt Road, Taipei, 10617 (Taiwan) (R.O.C.)
	E-mail: tswang@ntu.edu.tw
	E. Ekalestari
	Department of Chemistry & Biochemistry
	University of California, Los Angeles
	Los Angeles, CA 90095 (USA)
	Supporting information and the ORCID identification number(s) for
Ă	the author(s) of this article can be found under:
	http://dx.doi.org/10.1002/anie.201603921.

Angew. Chem. Int. Ed. 2016, 55, 1-6

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

### Wiley Online Library

These are not the final page numbers!

Enterobactin (Ent) is a catecholate siderophore secreted by Gram-negative enteric bacteria.<sup>[9]</sup> It is composed of an Lserine trilactone linked to three 2,3-dihydroxybenzoic acid (DHBA) moieties by amide bonds (Figure 1 a).<sup>[2b,10]</sup> The six hydroxyl groups on DHBA can form a hexadentate ligand for binding iron(III).<sup>[11]</sup> The Ent-iron(III) complex will be internalized by an outer membrane receptor, FepA,<sup>[12]</sup> and transported to the cytosol by FepBCDG.<sup>[4,13]</sup> Interestingly, Ent can be glucosylated by IroB at the C5 position of DHBA, producing monoglucosylated Ent (MGE) and diglucosylated Ent (DGE), also known as Salmochelin S4 (Figure 1 a).<sup>[14]</sup> The glucosylation does not compromise the iron binding.<sup>[15]</sup> The glucose (Glc) moiety provides additional water solubility compared to Ent.<sup>[14a]</sup> In K. pneumoniae, microcin E492 (Mcc E492, Figure 1b) uses its peptide moiety as an active toxin and Ent as a targeting probe against FepA-expressing strains.<sup>[16]</sup> Strikingly, the large peptide cargo can be carried through the outer membrane by the Ent-FepA uptake system.<sup>[16a,17]</sup> This implies that the MccE-492-like modifications do not compromise the internalization of Ent. We therefore hypothesized that using the same modification strategy on Ent, we should be able to transport other conjugated cargos into the bacteria.

Guided by MccE492, we aimed to devise a strategy for synthesizing MGE analogues with different functional groups on the C6 position of Glc for conjugation. The current method for obtaining MGE requires a nine-step synthesis of the glucosylated DHBA, and there is no evidence regarding whether the Glc-functionalized MGE can be made.<sup>[18]</sup> Moreover, the synthesis of nonglucosylated monofunctionalized Ent can be non-trivial.<sup>[7b]</sup> The complexity stems from the requirement of synthesizing the modified DHBA and the stochastic nature of linking two types of DHBA onto the trilactone. Herein, to circumvent the arduous synthesis, we developed a facile and versatile chemoenzymatic synthesis of Ent analogues with conjugatable groups, and then we demonstrated the feasibility of using Ent conjugates to target bacteria with selectivity.

We attempted to use the IroB enzyme to introduce Glc analogues to achieve functionalized microcin-like MGE (Figure 1 c). IroB is a glycosyltransferase that uses UDP-Glc (**2a**) to glucosylate Ent.<sup>[14b,19]</sup> To introduce Glc analogues, UDP-Glc analogues (**2a–d**) are needed as sugar donors. Although practical methods have been reported, it is not trivial to prepare UDP-sugars in large quantities. It has been shown that certain UDP-sugars can be made from 2-chloro-4nitrophenyl glycosides (CNP-sugars) by reversed glycosylation using OleD mutants.<sup>[20]</sup> We synthesized several CNPsugars (**1a–d**) for testing (Figure 1c).<sup>[20]</sup> To our surprise, wild-



*Figure 1.* Structures and synthesis of Ent analogues. a) IroB enzymatically modifies Ent at the C5 position of DHBA, producing MGE, DGE, or TGE. b) A truncated chemical structure of microcin E492. c) Left: Schematic of combined OleD and IroB enzymatic reactions starting from CNP-sugars to produce functionalized MGEs. Right: Designation of the compounds used in the reactions.

type OleD, cloned from *S. antibioticus* (ATCC11891), can readily catalyze the formation of **2a–d** from corresponding the CNP-Glc analogues (**1a–d**) in good yields, as judged by the yellow color of the released nitrophenate. HPLC and ESI-MS analysis of the reaction mixtures clearly showed the disappearance of UDP and emergence of UDP-Glc analogues, confirming the validity of the reaction (Supporting Information, Figure S1).

We then sought to combine the OleD reaction with the IroB glucosylation in one pot to afford functionalized MGE, bypassing the need to purify the UDP-sugars (Figure 1 c). Prior efforts have shown the feasibility of coupling a reverse glycosylation, which generates UDP-Glc in situ, to a second glycosylation.<sup>[20]</sup> After optimization, our working protocol for

a one-pot double-enzymatic conversion started with incubating **1a–d**, UDP, and OleD. Subsequently, without purifying the UDP-sugars, Ent and IroB were added directly to the mixtures. LC/MS analysis confirmed the formation of glycosylated Ent for all the CNP-Glc analogues (Figure 2 and the Supporting Information, Figure S2). In our test condition (Ent:CNP-sugar is 1:6), **1a** produces DGE ((Glc)<sub>2</sub>Ent) and triglucosylated Ent (TGE or (Glc)<sub>3</sub>-Ent), consistent with previous reports.<sup>[14b,21]</sup> **1b** produces mainly (6azGlc)<sub>2</sub>Ent and some (6azGlc)<sub>3</sub>Ent, while **1c** and **1d** produce mainly (6BrGlc)<sub>1</sub>Ent (**3c**) and (6HS-Glc)<sub>1</sub>Ent (**3d**), respectively (Supporting Information, Figure S2). The number of glucosylations can be tailored by adjusting the ratio of Ent and CNP-sugars, providing a convenient method to obtain tri, di,



Figure 2. LC/MS analysis of the combined OleD-IroB enzymatic reaction of CNP-6azGlc and Ent. Chromatograms and mass spectra of major Ent species are shown. The ratio of Ent and CNP-sugars used is shown in red at the top-left corner. a) For the test ratio 1:6, the major products are (6azGlc)<sub>3</sub>Ent and (6azGlc)<sub>2</sub>Ent. b) For the optimal ratio 1:3, the major product is (6azGlc)<sub>1</sub>Ent.

www.angewandte.org

2

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

or monoglucosylated products (Figure 2). Using HPLC, we are able to purify the products (Supporting Information, Figure S3). Currently, only small quantities (a few milligrams) can be obtained by a single reaction. Although the scale was sufficient for preliminary tests, practical larger-scale production would demand further improvements in reaction conditions and separation. Toward this end, we have demonstrated a facile double enzymatic reaction to obtain a family of functionalized MGEs, such as  $(6azGlc)_1Ent$  (**3b**), **3c**, and **3d** (Figure 1 c). These versatile functional groups can provide synthetic flexibility in further applications.

We next evaluated the conjugation reactions with the functionalized MGEs (**3b-d**). Azido-functionalized **3b** was reacted with rhodamine B-alkyne (RhB-alkyne, **26**) using a convenient copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction and purified by HPLC to afford RhB-Glc-Ent (Figure 3a). Thiol-functionalized **3d** can be linked to a dansyl group by a disulfide bond exchange using 2-mercaptopyridine activated reactant **29** (Dansyl-PDS) to afford Dan-Glc-Ent (Figure 3b and the Supporting Information, Figure S5 and Scheme S10). However, bromo-functionalized **3c** did not react with dansylthiol (**28**) in any condition tested, and not even with 2-mercaptoethanol, owing probably to the hindered environment around C6-Br, on which the substitution takes place.



**Figure 3.** Synthesis of a) RhB-Glc-Ent through CuAAC and b) Dan-Glc-Ent through disulfide bond exchange. c) Microscopy images of *E. coli* CFT073 treated with RhB-Glc-Ent (top) and Dan-Glc-Ent (bottom). Left: bright field (BF), middle: fluorescence channel, right: merge.

Fluorescently labeled Ent that targets bacteria can be useful for detecting bacteria. To confirm the capacity of Ent conjugates to target bacteria, we treated E. coli CFT073 with RhB-Glc-Ent and Dan-Glc-Ent, and examined the treated bacteria by fluorescent microscopy. CFT073 was incubated in M63 minimal medium to provide an iron-limiting environment. Whereas the RhB-Glc-Ent and Dan-Glc-Ent treated groups were mostly fluorescently labeled, the 26- and dansyl disulfide 27-treated control groups showed no fluorescent bacteria (Figure 3c and Supporting Information, Figure S6). Some bacteria were only weakly labeled by our probes, which could result from the heterogeneity of protein expression in the population.<sup>[22]</sup> Other possibilities for weak or no labeling cannot be ruled out at this time and further characterization is required. Nevertheless, heterogeneous labeling was frequently observed with other probes, including siderophorebased probes.<sup>[23]</sup> We further confirmed the labeling by flow cytometry. When 10 µM RhB-Glc-Ent was added, a significant 3-fold increase in fluorescence intensity was observed after 1 h, and it continued to increase to 6-fold over 7 h. In contrast, when 10 µM 26 was treated as a control, the increase was less than 2-fold even over an extended time period (Supporting Information, Figures S7 and S8). Our combined results clearly indicate that the Ent portion contributed to the higher fluorescence increase, owing to the Ent uptake machinery.

We expanded our experiment to other strains to examine the selectivity, including E. coli CFT073, K12, O157:H7; P. aeruginosa; V. cholerae; S. aureus; and B. subtilis (Figure 4). The E. coli strains and P. aeruginosa were previously reported to use Ent,<sup>[7b,24]</sup> whereas V. cholerae uses linearized Ent but not cyclic Ent.<sup>[25]</sup> The Gram-positive S. aureus and B. subtilis use other siderophores and were not reported to take up glucosylated Ent.<sup>[26]</sup> In the minimal medium, the two Grampositive bacteria did not take up RhB-Glc-Ent. All three E. coli strains and P. aeruginosa showed an over 5-fold fluorescence increase when treated with RhB-Glc-Ent (Figure 4a and the Supporting Information, Figure S9). However, although V. cholerae was reported not to take up cyclic Ent, RhB-Glc-Ent treatment resulted in a weaker increase. One possibility is the low usage of cyclic Ent by V. cholerae, as observed in the literature.<sup>[27]</sup> To further explore whether the probe targeting is iron dependent, we cultured all bacteria in an iron-rich medium. The bacteria tested showed no significant fluorescence increase (Figure 4b and the Supporting Information, Figure S10). The combined results demonstrated that the uptake of RhB-Glc-Ent is dependent on culture conditions in E. coli, P. aeruginosa, and V. cholerae. To rule out the possibility of other factors in LB rich medium, we cultured CFT073 in M63 minimal medium supplemented with 100 µM FeCl<sub>3</sub> (Figure 4b and the Supporting Information, Figure S11). Compared to the M63 minimal medium, CFT073 showed no significant fluorescence increase in the iron-rich environment of M63 + FeCl<sub>3</sub>. This again supports the conclusion that RhB-Glc-Ent targeting is indeed sensitive to iron conditions.

In conclusion, we have provided a convenient chemoenzymatic synthesis of functionalized MGE that would facilitate the application of siderophore conjugates. Although many siderophore conjugates have been developed, the

Angew. Chem. Int. Ed. 2016, 55, 1-6

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

www.angewandte.org

Communications



**Figure 4.** Bacterial labeling with 10  $\mu$ M RhB-Glc-Ent. Bars represent average of the medium of fluorescence intensity of bacteria measured by flow cytometry in triplicate. (+): treated with RhB-Glc-Ent, (-): bacteria alone. a) Bacteria cultured in minimal medium. b) Bacteria in LB. Inset: *E. coli* CFT073 cultured in M63 minimal medium supplemented with 100  $\mu$ M FeCl<sub>3</sub>. (statistical analysis: \*\*\* as p < 0.001, n.s. as not significant, n=3 for all tests).

scaffold is often a synthetic mimic rather than a natural structure.<sup>[7e,f,8]</sup> This could potentially result in altered molecular recognition and give perplexing results.<sup>[7f]</sup> The natural scaffold of our functionalized MGE ensures that the conjugate will conserve its targeting selectivity better than the synthetic scaffolds, and therefore be more competitive with native siderophores.

In preparing functionalized MGE, we employed a double enzymatic reaction using OleD and IroB to circumvent a tedious chemical synthesis. Although recent efforts successfully modified monofunctionalized Ent with IroB to afford only monofunctionalized MGE or DGE,<sup>[7c]</sup> the third Glc modification site was blocked, whereas our chemoenzymatic functionalization can potentially achieve a trifunctionalized TGE using native Ent. This approach allows us to explore more possibilities, as the number of Glc moieties can affect the strain selectivity.<sup>[28]</sup>

Functionalized MGE, when linked with a fluorophore, acts as a targeting probe for selective bacterial detection. Notably, labeling of the bacteria is dependent on both species and iron availability. Although this limits the use of Ent conjugates to only iron-limiting conditions, it does provide selectivity towards bacteria in iron-limiting environments. In mammalian hosts, free iron is extremely scarce and bacteria would rely on a siderophore pathway to obtain iron.<sup>[1a]</sup> This makes the bacteria in hosts a great target for Ent conjugates, while rendering bacteria in non-host, iron-rich environments free of targeting.

The current limitation of our method is the reaction scale. An improvement in making Ent conjugates is required for practical use. Another limitation is the dependency of our targeting probes on the activity of the iron-acquisition pathway, which could lead to heterogeneity in targeting efficiency. While this makes it less potent as a universal method, relying on siderophore pathway enables the method to perform unique applications, such as iron-limited specific labeling during host infection and cargo delivery into bacteria inside hosts. We believe that these applications should not be over-shadowed by the limitations.

#### Acknowledgements

We thank the Ministry of Science and Technology, Taiwan, (MOST 104-2119-M-002-021- and NSC 103-2113-M-002-001-MY2) and Center for Emerging Material and Advanced Devices, National Taiwan University (NTU) (NTU-ERP-105R8956-4) for financial support. We also thank Dr. Ching-Hao Teng at National Cheng Kung University for providing *E. coli* CFT073, and Dr. Ya-Wen Liu at School of Medicine, NTU for providing the fluorescent microscope. Finally, we thank the Proteomics MS Core Facility and Chemical Biology Core Facility at Department of Chemistry, NTU, for instrumental support.

**Keywords:** bacteria detection · chemoenzymatic synthesis · click chemistry · enterobactin · siderophores

- a) M. Miethke, M. A. Marahiel, *Microbiol. Mol. Biol. Rev.* 2007, 71, 413–451; b) J. B. Neilands, *J. Biol. Chem.* 1995, 270, 26723– 26726; c) R. C. Hider, X. L. Kong, *Nat. Prod. Rep.* 2010, 27, 637– 657.
- [2] a) K. Hantke, *Curr. Opin. Microbiol.* 2001, *4*, 172–177; b) J. H. Crosa, C. T. Walsh, *Microbiol. Mol. Biol. Rev.* 2002, *66*, 223–249.
- [3] M. L. Guerinot, Annu. Rev. Microbiol. 1994, 48, 743-772.
- [4] K. D. Krewulak, H. J. Vogel, *Biochim. Biophys. Acta Biomembr.* 2008, 1778, 1781–1804.
- [5] a) R. C. Hider, Struct. Bonding (Berlin) 1984, 58, 25–87; b) G.
   Winkelmann, Biochem. Soc. Trans. 2002, 30, 691–696.
- [6] a) V. Braun, A. Pramanik, T. Gwinner, M. Koberle, E. Bohn, *Biometals* 2009, 22, 3–13; b) U. Möllmann, L. Heinisch, A. Bauernfeind, T. Kohler, D. Ankel-Fuchs, *Biometals* 2009, 22, 615–624.
- [7] a) M. G. Page, Ann. N. Y. Acad. Sci. 2013, 1277, 115-126; b) T. Zheng, E. M. Nolan, J. Am. Chem. Soc. 2014, 136, 9677-9691;
  c) P. Chairatana, T. Zheng, E. M. Nolan, Chem. Sci. 2015, 6, 4458-4471; d) J. M. Roosenberg II, Y. M. Lin, Y. Lu, M. J. Miller, Curr. Med. Chem. 2000, 7, 159-197; e) C. Ji, P. A. Miller, M. J. Miller, J. Am. Chem. Soc. 2012, 134, 9898-9901; f) A. P. Tomaras, J. L. Crandon, C. J. McPherson, M. A. Banevicius,

www.angewandte.org

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Angew. Chem. Int. Ed. 2016, 55, 1-6

These are not the final page numbers!

S. M. Finegan, R. L. Irvine, M. F. Brown, J. P. O'Donnell, D. P. Nicolau, *Antimicrob. Agents Chemother.* **2013**, *57*, 4197–4207.

- [8] a) T. Inomata, H. Eguchi, Y. Funahashi, T. Ozawa, H. Masuda, *Langmuir* 2012, 28, 1611–1617; b) D. D. Doorneweerd, W. A. Henne, R. G. Reifenberger, P. S. Low, *Langmuir* 2010, 26, 15424–15429.
- [9] K. N. Raymond, E. A. Dertz, S. S. Kim, Proc. Natl. Acad. Sci. USA 2003, 100, 3584–3588.
- [10] a) A. M. Gehring, K. A. Bradley, C. T. Walsh, *Biochemistry* 1997, 36, 8495–8503; b) A. M. Gehring, I. Mori, C. T. Walsh, *Biochemistry* 1998, 37, 2648–2659; c) C. A. Shaw-Reid, N. L. Kelleher, H. C. Losey, A. M. Gehring, C. Berg, C. T. Walsh, *Chem. Biol.* 1999, 6, 385–400; d) D. E. Ehmann, C. A. Shaw-Reid, H. C. Losey, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* 2000, 97, 2509–2514.
- [11] a) W. R. Harris, C. J. Carrano, S. R. Cooper, S. R. Sofen, A. E. Avdeef, J. V. McArdle, K. N. Raymond, *J. Am. Chem. Soc.* **1979**, *101*, 6097–6104; b) L. D. Loomis, K. N. Raymond, *Inorg. Chem.* **1991**, *30*, 906–911.
- [12] a) S. M. Newton, J. D. Igo, D. C. Scott, P. E. Klebba, *Mol. Microbiol.* 1999, *32*, 1153–1165; b) S. K. Buchanan, B. S. Smith, L. Venkatramani, D. Xia, L. Esser, M. Palnitkar, R. Chakraborty, D. van der Helm, J. Deisenhofer, *Nat. Struct. Biol.* 1999, *6*, 56–63; c) J. D. Faraldo-Gómez, M. S. Sansom, *Nat. Rev. Mol. Cell Biol.* 2003, *4*, 105–116.
- [13] a) D. L. Stephens, M. D. Choe, C. F. Earhart, *Microbiology* 1995, 141, 1647–1654; b) C. M. Shea, M. A. Mcintosh, *Mol. Microbiol.* 1991, 5, 1415–1428; c) S. S. Chenault, C. F. Earhart, *Mol. Microbiol.* 1991, 5, 1405–1413.
- [14] a) B. Bister, D. Bischoff, G. J. Nicholson, M. Valdebenito, K. Schneider, G. Winkelmann, K. Hantke, R. D. Süssmuth, *Biometals* 2004, *17*, 471–481; b) M. A. Fischbach, H. Lin, D. R. Liu, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* 2005, *102*, 571–576.
- [15] K. Hantke, G. Nicholson, W. Rabsch, G. Winkelmann, Proc. Natl. Acad. Sci. USA 2003, 100, 3677–3682.

- [16] a) G. Mercado, M. Tello, M. Marin, O. Monasterio, R. Lagos, J. Bacteriol. 2008, 190, 5464–5471; b) V. de Lorenzo, A. P. Pugsley, Antimicrob. Agents Chemother. 1985, 27, 666–669.
- [17] E. Strahsburger, M. Baeza, O. Monasterio, R. Lagos, Antimicrob. Agents Chemother. 2005, 49, 3083–3086.
- [18] X. Yu, Y. Dai, T. Yang, M. R. Gagné, H. Gong, *Tetrahedron* 2011, 67, 144–151.
- [19] A. J. Bäumler, R. M. Tsolis, A. W. van der Velden, I. Stojiljkovic, S. Anic, F. Heffron, *Gene* 1996, 183, 207–213.
- [20] R. W. Gantt, P. Peltier-Pain, W. J. Cournoyer, J. S. Thorson, *Nat. Chem. Biol.* 2011, 7, 685–691.
- [21] D. Foshag, C. Campbell, P. D. Pawelek, Biochim. Biophys. Acta Proteins Proteomics 2014, 1844, 1619–1630.
- [22] a) Y. Taniguchi, P. J. Choi, G. W. Li, H. Chen, M. Babu, J. Hearn, A. Emili, X. S. Xie, *Science* 2010, *329*, 533-538; b) C. A. Strassert, M. Otter, R. Q. Albuquerque, A. Höne, Y. Vida, B. Maier, L. De Cola, *Angew. Chem. Int. Ed.* 2009, *48*, 7928-7931; *Angew. Chem.* 2009, *121*, 8070-8073.
- [23] S. Noël, L. Guillon, I. J. Schalk, G. L. Mislin, Org. Lett. 2011, 13, 844–847.
- [24] K. Poole, L. Young, S. Neshat, J. Bacteriol. 1990, 172, 6991-6996.
- [25] E. E. Wyckoff, B. E. Allred, K. N. Raymond, S. M. Payne, J. Bacteriol. 2015, 197, 2840–2849.
- [26] a) M. T. Sebulsky, D. E. Heinrichs, J. Bacteriol. 2001, 183, 4994–5000; b) A. M. Zawadzka, R. J. Abergel, R. Nichiporuk, U. N. Andersen, K. N. Raymond, Biochemistry 2009, 48, 3645–3657.
- [27] G. L. Griffiths, S. P. Sigel, S. M. Payne, J. B. Neilands, J. Biol. Chem. 1984, 259, 383-385.
- [28] G. J. Williams, C. Zhang, J. S. Thorson, *Nat. Chem. Biol.* **2007**, *3*, 657–662.

Received: April 22, 2016 Revised: July 7, 2016 Published online:

These are not the final page numbers! 77

www.angewandte.org



### Communications



## Communications

#### Siderophore Conjugates

A. A. Lee, Y.-C. S. Chen, E. Ekalestari, S.-Y. Ho, N.-S. Hsu, T.-F. Kuo, T.-S. A. Wang\* \_\_\_\_\_\_

Facile and Versatile Chemoenzymatic Synthesis of Enterobactin Analogues and Applications in Bacterial Detection



**Siderophore-based bacteria labeling**: A one-pot chemoenzymatic synthesis approach that is capable of functionalizing enterobactin with different reactive groups is developed. The functionalized

enterobactin can be further conjugated with fluorophores to perform specific detection of bacteria. This strategy can serve as a convenient way to deliver cargos into bacteria with selectivity.

6 www.angewandte.org

These are not the final page numbers!