

# Concise Synthesis of Calystegine B<sub>2</sub> and B<sub>3</sub> via Intramolecular Nozaki–Hiyama–Kishi Reaction

Received 00th January 20xx,  
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Hong-Yao Wang,<sup>a,e</sup> Atsushi Kato,<sup>\*b</sup> **Kyoko Kinami,<sup>b</sup>** Yi-Xian Li,<sup>a</sup> Yue-Mei Jia,<sup>a</sup> George W. J. Fleet,<sup>c,d</sup> and Chu-Yi Yu<sup>\*a,d</sup>

A concise method for the synthesis of calystegine B<sub>2</sub> and its C-2 epimer calystegine B<sub>3</sub> was developed starting from the readily available sugar-derived materials. The key feature of this synthetic route was the construction of the cycloheptanone ring via intramolecular Nozaki–Hiyama–Kishi (NHK) reaction, which afforded the key intermediate cycloheptanone **8** in excellent yield. The precursor for the intramolecular NHK reaction, aldehyde **9** with a Z-vinyl iodide functional group, was synthesized through Stork olefination of aldehyde **10**, which was derived from 2,3,4-tri-O-benzyl-D-xylopyranose **11**. Calystegine B<sub>2</sub> (**3**) and calystegine B<sub>3</sub> (**4**) were synthesized in 11 steps in excellent overall yields (27% and 19%).

## Introduction

Calystegines are an intriguing class of polyhydroxylated iminosugar alkaloids with a nortropane skeleton, which were initially isolated in 1988 from *Calystegia sepium*<sup>1</sup>. These nortropane iminosugars<sup>2</sup> were later found widely in various vegetables and fruits, such as potatoes, tomatoes, aubergines and cabbages<sup>3</sup>. The naturally-occurring calystegines have been classified into three types according to the number of hydroxyl groups on the nortropane skeleton<sup>4</sup>: A (with three OH groups), B (with four OH groups) and C (with five OH groups). Apart from these three basic types of calystegines, a novel type of nortropane iminosugar with a bridgehead amino group was also isolated and named as calystegine N<sub>1</sub><sup>5</sup>, which was an interesting addition to the calystegine family (Figure 1).

Calystegines exhibited potent and selective glycosidase inhibition<sup>6</sup>, particularly against glucosidases and galactosidases<sup>3, 5a, 6-7</sup>; they also showed great potential in the treatment of cancer<sup>8</sup>, viral infections<sup>9</sup>, diabetes<sup>10</sup>, tuberculosis<sup>11</sup> and glycosphingolipid storage disorders<sup>12</sup>. Due to their intriguing chemical structures and important biological activities, the synthesis of calystegines have attracted much

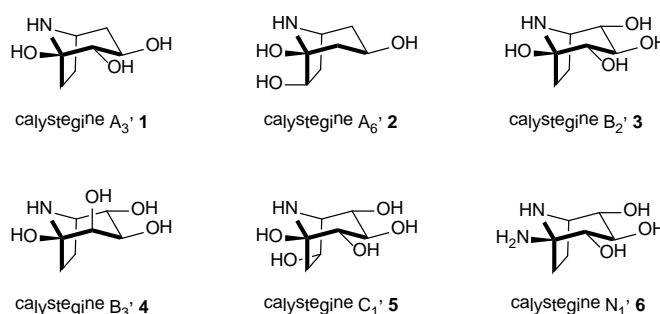


Figure 1 Examples of naturally occurring calystegines

attention ever since they were isolated; a number of synthetic strategies<sup>13</sup> have so far been developed for the preparation of calystegines. Representative methods for the synthesis of calystegines include ring closing metathesis (RCM)<sup>13a-g</sup>, ring expansions<sup>13h-j</sup>, cycloadditions<sup>13k-m</sup>, radical cyclization<sup>13n</sup> and polar cyclization<sup>13o, 13p</sup>. Furthermore, various derivatives and analogues of calystegines<sup>14</sup>, such as nojiristegine<sup>14a</sup>, labstegine<sup>14b</sup>, and fluorinated derivatives<sup>14c</sup> were also designed and synthesized to explore the structure–activity relationship of calystegines. Among these syntheses, RCM reaction enjoyed popularity due to its efficiency and generality in the construction of the cycloheptanone ring which is the key step in many syntheses of calystegines. However, one of the major drawbacks with the RCM strategy was that the resulting cycloheptene needed further modifications to be transformed into the properly functionalized cycloheptanone; poor regioselectivity<sup>13a-g</sup> in the installation of the hydroxyl group by the hydroboration-oxidation reaction generally gave mixtures of regioisomers of the cycloheptanols.

<sup>a</sup> Beijing National Laboratory for Molecular Science (BNLMS), CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. E-mail: yucy@iccas.ac.cn

<sup>b</sup> Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. E-mail: kato@med.u-toyama.ac.jp

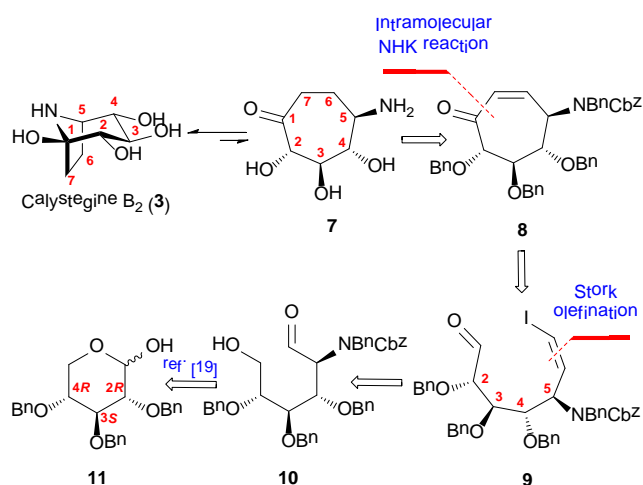
<sup>c</sup> Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK.

<sup>d</sup> National Engineering Research Center for Carbohydrate Synthesis, Jiangxi Normal University, Nanchang 330022, PR China

<sup>e</sup> University of Chinese Academy of Sciences, Beijing 100049, China.

Electronic Supplementary Information (ESI) available: Copies of <sup>1</sup>H, <sup>13</sup>C and 2D NMR spectra, data of crystal structure and results of the bioassay. See DOI: 10.1039/x0xx00000x

In the context of our interest in the synthesis and biological activities of iminosugars<sup>15</sup>, we became interested in the study of the biological activity of the calystegines and the design of more efficient strategies and better synthetic methods. In order to develop a general and efficient method for the synthesis of calystegines, we chose calystegine B<sub>2</sub> (**3**) and its C2 epimer calystegine B<sub>3</sub> (**4**) as the initial targets. Calystegine B<sub>2</sub> (**3**) was isolated from *Calystegia sepium*<sup>1</sup>. It was chosen as the target compound because it possessed the typical polyhydroxylated nortropane structure of calystegines and has potent and highly selective inhibition of  $\beta$ -glucocerebrosidase, the enzyme involved in Gaucher disease<sup>7a,16</sup>. Additionally, its C-2 epimer, i.e. calystegine B<sub>3</sub> (**4**) extracted from *Physalis alkekengi* var. *francheti*<sup>2d</sup> was selected to verify the generality of our method.



**Scheme 1** Retrosynthetic analysis of calystegine B<sub>2</sub>.

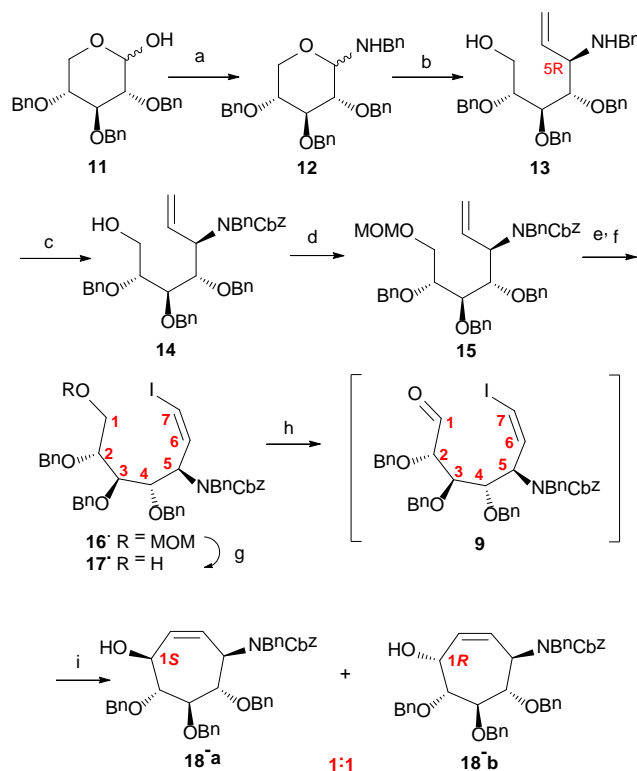
Our retrosynthetic analysis is outlined in Scheme 1. As has been reported by others, the tetrahydroxylated nortropane structure of calystegine B<sub>2</sub> was the more stable bicyclic tautomer of 2,3,4-trihydroxyl-5-amino-cycloheptanone **7** which spontaneously cyclized to the more stable nortropane form<sup>13</sup>. Cycloheptanone **7** could be obtained by hydrogenolysis of 2,3,4-tribenzyloxy-5-amino-cycloheptenone **8**. Therefore, the key task in the synthesis is the construction of the key intermediate cycloheptenone **8** from aldehyde **9** through an intramolecular Nozaki–Hiyama–Kishi (NHK) reaction. The NHK reaction was found to be compatible with a wide range of sensitive functional groups and had been employed in the synthesis of some complex natural products<sup>17</sup>. However, to the best of our knowledge, it has not been used previously in the synthesis of either polyhydroxylated cycloheptenones or calystegines. The aldehyde **9** with a Z-vinyl iodide group at C-5 could be prepared *via* Stork's olefination<sup>18</sup> of  $\alpha$ -amino-aldehyde **10**, readily obtained from 2,3,4-tri-O-benzyl-D-xylopyranose **11** through a series of well-documented transformations<sup>19</sup>. There are some obvious merits of this synthetic method. First, the D-xylose derived starting material **11** is readily available and contains three hydroxyl groups with

the correct stereochemistry (C2, C3 and C4) required for the construction of calystegine B<sub>2</sub> (**3**); secondly, the key intermediate, 2,3,4-trihydroxyl-5-amino-cycloheptenone **8**, contains all the required functional groups with the correct configurations to afford the target compound **3** by removal of the protecting groups without any other chemical transformation; thirdly, this method could become a general method for the synthesis of calystegines and their stereoisomers simply by replacement of the protected xylose **11** with those derived from other pentoses.

## Results and discussion

### Synthesis of calystegine B<sub>2</sub>

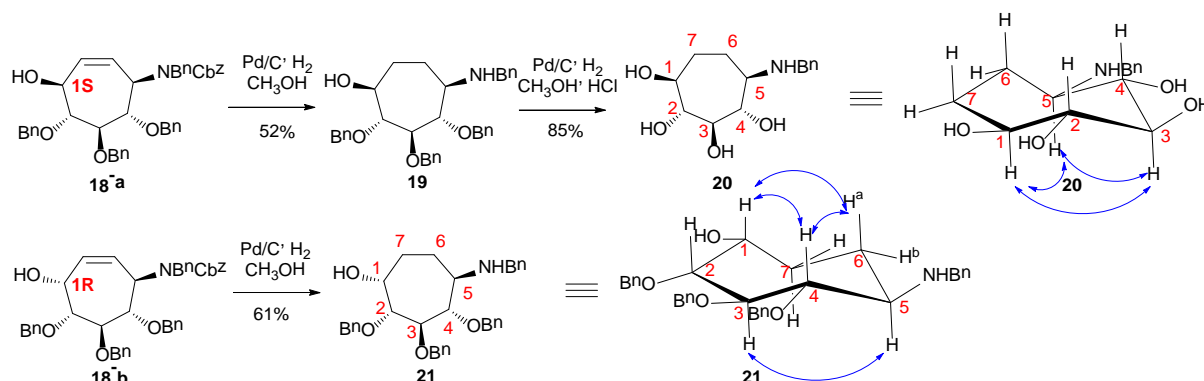
2,3,4-Tri-O-benzyl-D-xylopyranose **11**, prepared from D-xylose in three steps (Scheme 2),<sup>20</sup> was converted to the *N*-benzyl glycosyl amine **12** *via* the reported method.<sup>21</sup> Vinyl Grignard addition to the glycosyl



**Scheme 2** Reagents and conditions: (a) BnNH<sub>2</sub>, pTsOH, DCM, rt, 87%; (b) CH<sub>2</sub>=CHMgCl, THF, 0°C, 89%; (c) CbzCl, NaHCO<sub>3</sub>, THF-H<sub>2</sub>O, 93%; (d) MOMCl, DIPEA, DCM, rt, 90%; (e) O<sub>3</sub>, DCM, Me<sub>2</sub>S; (f) PPh<sub>3</sub>=CHI, NaHMDS, THF, 80% for 2 steps; (g) 1N HCl, MeOH, rt, 96%; (h) COCl<sub>2</sub>, DMSO, DCM, Et<sub>3</sub>N; (i) CrCl<sub>2</sub>, NiCl<sub>2</sub>, DMF, rt, 78% for 2 steps.

## Organic &amp; Biomolecular Chemistry

## ARTICLE

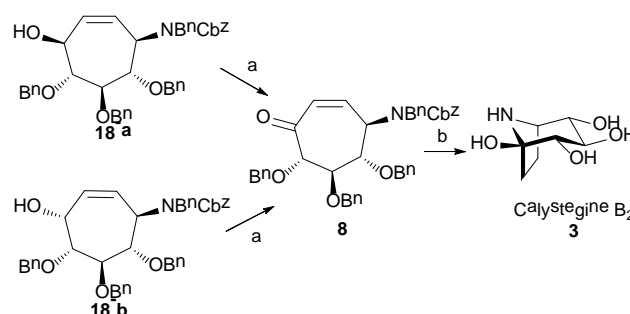
Scheme 3 The NOESY of **20** and **21**

amine **12** provided allyl amine **13** as the single diastereomer<sup>19</sup>. The high diastereoselectivity was in accordance with previous reports and could be explained with a Cram-chelate model<sup>19</sup>. The allyl amine **13** was then protected with Cbz group to give the carbamate **14**. However, the subsequent ozonolysis of the carbamate **14** and Stork's olefination<sup>18b</sup> unexpectedly resulted in the formation of a complicated mixture of products; none of the desired Z-vinyl iodide **17** was observed. The free hydroxyl group in compound **14** may be the source of the problem since it could attack the Cbz group under basic conditions. Thus, *O*-MOM protection of **14** gave the allyl amine **15**. Ozonolysis of **15** followed by Stork's olefination produced the Z-vinyl iodide **16** in 80% yield in two steps. Removal of the *O*-MOM protecting group in **16** with hydrochloric acid afforded compound **17** in 96% yield. The Z-geometry of the newly formed carbon-carbon double bond in **17** was confirmed by the  $J_{H6,H7}$  value (7.2 Hz) in the <sup>1</sup>H-NMR.

With Z-vinyl iodide **17** in hand, we attempted synthesis of the cycloheptenol **18** via intramolecular NHK reaction (Scheme 2). Swern oxidation of **17** gave aldehyde **9**, the substrate of the intramolecular NHK reaction, *in situ* and directly used in the next step due to its chemical instability. After treatment of compound **9** with a combination of 10 equiv of CrCl<sub>2</sub> and catalytic amounts (5 mol %) of NiCl<sub>2</sub> in DMF in highly-diluted solution at room temperature for 30 h, the intramolecular NHK reaction of **9** took place and provided the expected product cycloheptenol **18** as a mixture of two separable diastereomers (**18a** and **18b**) in an approximate ratio of 1:1. The poor diastereoselectivity was not a significant problem as both these two isomers were to be transformed into the same cycloheptenone after oxidation of the C1 hydroxyl group.

It was difficult to identify the relative C1 configuration of **18-a** and **18-b** due to the multiple signals caused by the *N*-Cbz rotamers. Thus, the Cbz protecting group of **18-a** and **18-b** was

removed *via* hydrogenolysis to afford the compound **19** and **21**, respectively (scheme 3). However, overlapping <sup>1</sup>H-NMR signals were observed for compound **19** so that further debenzoylation was conducted by further hydrogenolysis to furnish **20**, which gave sharp <sup>1</sup>H-NMR signals. The *S*-configuration of C1 in **18-a** was unambiguously established on the basis of NOESY experiments of **20**, which showed strong interactions between H1 and H3, H1 and H5. Accordingly, the C1 configuration of **21** was determined as *R*-configuration by the strong interactions between H1 and H4, H1 and H6a. Unlike <sup>1</sup>H-NMR of **19**, <sup>1</sup>H-NMR of **21** showed well resolved signals.



Scheme 4 Total synthesis of calystegine B<sub>2</sub>. Reagents and conditions: (a) DMP, NaHCO<sub>3</sub>, DCM, rt, 91%; (b) Pd/C, H<sub>2</sub>, CH<sub>3</sub>COOH, 81%.

As was expected, Dess-Martin periodinane (DMP) oxidation of **18-a** and **18-b** gave the same cycloheptenone **8**. Then, Pd-catalyzed hydrogenation of **8** in acetic acid<sup>13g</sup> afforded calystegine B<sub>2</sub> (**3**) in excellent yield (Scheme 4). The compound was fully characterized by NMR analysis and the data were consistent with those reported for the natural product. Furthermore, the optical rotation of synthetic calystegine B<sub>2</sub> (**3**)

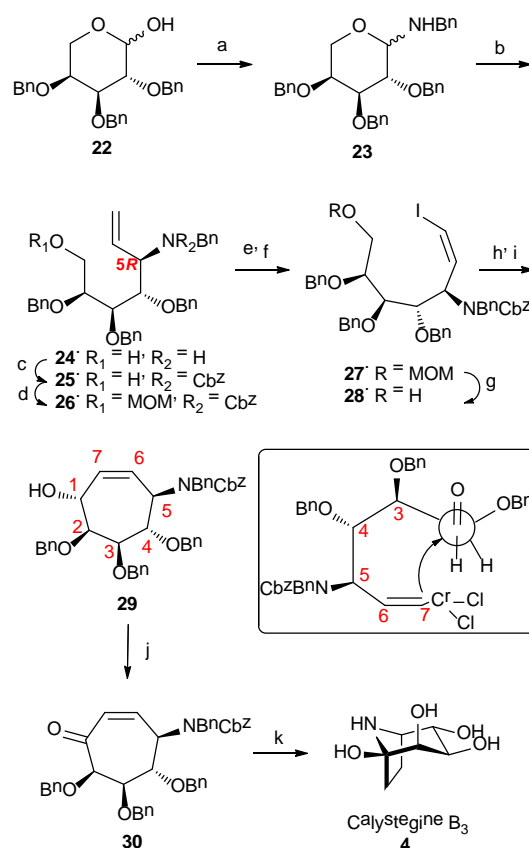
( $[\alpha]_{D_{20}} +28.0$ ,  $c$  0.5 in  $H_2O$ ) matched well with that reported for the natural product<sup>2d</sup> ( $[\alpha]_{D_{20}} +27.2$ ,  $c$  0.5 in  $H_2O$ ).

[the rotations in the paper should be subscript D and superscript 20]

#### Synthesis of calystegine B<sub>3</sub>

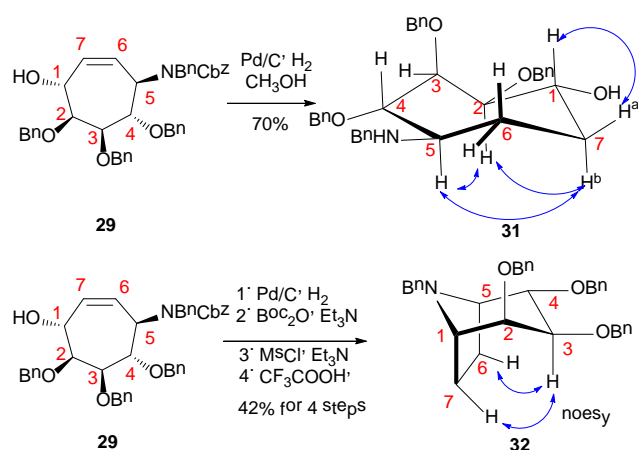
The synthesis of calystegine B<sub>3</sub> (**4**) by the same strategy as above for calystegine B<sub>2</sub> (**3**) showed the generality of the approach to calystegines. The configurations of C2, C3 and C4 in calystegine B<sub>3</sub> (**4**) identified 2,3,4-tri-*O*-benzyl-L-arabinopyranose **22** (Scheme 5), readily prepared from L-arabinose<sup>20</sup> as the appropriate starting material for a similar synthesis of calystegine B<sub>3</sub> (**4**). The *N*-benzyl glycosyl amine **23** was obtained in 70% yield. Vinyl Grignard addition of **23** gave vinyl amine **24** as a single diastereomer with 5*R* configuration. After protection of the amine **24** with Cbz group, the resulting alcohol **25** was treated with MOMCl to afford the fully protected **26**. Ozonolysis of compound **26** followed by Stork's olefination furnished *Z*-vinyl iodide **27** in 75% yield over 2 steps. The MOM group was easily removed with hydrochloric acid to provide compound **28**. In contrast to the intramolecular NHK reaction in the synthesis of calystegine B<sub>2</sub> (**3**), cyclization of **28** resulted only in one diastereomer **29**. This result may be rationalized by a Felkin–Anh transition state<sup>17f, 17g, 22</sup>, which is known to be the preferred pathway in additions of organochromium reagents to aldehydes (Scheme 5). Oxidation of **29** with Dess–Martin periodinane (DMP) gave the required cycloheptanone **30** in 93% yield and then hydrogenolysis of **30** in acetic acid<sup>13g</sup> afforded calystegine B<sub>3</sub> (**4**) in good yield (80%).

The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the synthetic calystegine B<sub>3</sub> (**4**) were identical to those reported for the natural product. The optical rotation of the synthetic calystegine B<sub>3</sub> ( $[\alpha]_{D_{20}} +68.0$ ,  $c$



**Scheme 5** Total synthesis of calystegine B<sub>3</sub>. Reagents and conditions: (a)  $BnNH_2$ ,  $pTsOH$ , DCM, rt, 70%; (b)  $CH_2=CHMgCl$ , THF, 0°C, 85%; (c)  $CbzCl$ ,  $NaHCO_3$ , THF- $H_2O$ , 90%; (d)  $MOMCl$ ,  $DIPEA$ , DCM, rt, 92%; (e)  $O_3$ , DCM,  $Me_2S$ ; (f)  $PPh_3=CHI$ ,  $NaHMDS$ , 75% for 2 steps; (g) 1N  $HCl$ ,  $MeOH$ , rt, 96%; (h)  $COCl_2$ ,  $DMSO$ , DCM,  $Et_3N$ ; (i)  $CrCl_2$ ,  $NiCl_2$ ,  $DMF$ , rt, 70% for 2 steps; (j)  $DMP$ ,  $NaHCO_3$ , DCM, rt, 93%; (k)  $Pd/C$ ,  $H_2$ ,  $CH_3COOH$ , 80%. 0.5 in  $H_2O$ ) is in accord with the literature values ( $[\alpha]_{D_{20}} +82.8$ ,  $c$  0.5 in  $H_2O$ )<sup>2d</sup>.

The C1 configuration of **29** was determined as *R* from the NOESY of **31** (strong interaction between H-1 and H-7a) which was obtained by removal of the Cbz group by hydrogenolysis (Scheme 6). Further supporting evidence for the stereochemical assignments came from the bicyclic compound **32**, which was derived from **29** through intramolecular  $S_N2$  cyclization between C1 and C5.

Scheme 6 The NOESY of **31** and **32**

The synthetic calystegine B<sub>2</sub> (**3**) was assayed as a potential glycosidase inhibitor of a range of enzymes and showed potent inhibition of human lysosome  $\beta$ -glucosidase ( $IC_{50}$  = 5.1  $\mu$ M) and coffee beans  $\alpha$ -galactosidase ( $IC_{50}$  = 3.2  $\mu$ M). In contrast, calystegine B<sub>3</sub> (2-*epi*-calystegine B<sub>2</sub>) showed much weaker inhibition against these enzymes. Recently, calystegine B<sub>3</sub> was identified as an inhibitor of cytoplasmic  $\alpha$ -mannosidase. Although it did not change the composition of cell-surface oligosaccharides, it caused a drastic change in both structure and quantity of free oligosaccharides in the cytosol<sup>7b</sup>. This study revealed that calystegine B<sub>3</sub> did not affect the plant enzyme Jack bean  $\alpha$ -mannosidase.

Table 1. Concentration of Calystegine B<sub>2</sub> (**3**) giving 50% inhibition of various glycosidases

enzyme	IC <sub>50</sub> ( $\mu$ M)	
	Calystegine B <sub>2</sub> , <b>3</b>	Calystegine B <sub>3</sub> , <b>4</b>
$\alpha$ -Glucosidase		
Yeast	NI <sup>a</sup> (3.2 %) <sup>b</sup>	NI (14.6 %)
Rice	NI (33.5 %)	154
Rat intestinal maltase	NI (25.1 %)	55
$\beta$ -Glucosidase		
Almond	5.3	84
Human lysosome	5.1	616
$\alpha$ -Galactosidase		
Coffee beans	3.2	73
$\beta$ -Galactosidase		
Bovine liver	383	71
$\alpha$ -Mannosidase		
Jack bean	NI (0 %)	NI (6.6 %)
$\beta$ -Mannosidase		
Snail	NI (0 %)	NI (2.0 %)
$\alpha$ -L-Fucosidase		
Bovine kidney	NI (0.1 %)	NI (2.7 %)
$\alpha$ , $\alpha$ -Trehalase		
Porcine kidney	19	384
Amyloglucosidase		
<i>Aspergillus niger</i>	NI (0 %)	NI (8.2 %)
$\alpha$ -L-Rhamnosidase		
<i>Penicillium decumbens</i>	NI (47.7 %)	NI (13.4 %)
$\beta$ -Glucuronidase		
<i>E. coli</i>	NI (6.9 %)	NI (8.8 %)
Bovine liver	NI (0 %)	NI (4.1 %)

<sup>a</sup> NI : no inhibition (less than 50% inhibition at 1000  $\mu$ M).

<sup>b</sup> ( ) : inhibition at 1000  $\mu$ M.

## Conclusion

In summary, via a concise and efficient route from the readily available sugar-derived materials, calystegine B<sub>2</sub> (**3**) and B<sub>3</sub> (**4**) were synthesized in 11 steps in excellent overall yields (27% and 19%, respectively). This method features an intramolecular NHK reaction and Stork olefination as key steps. To the best of our knowledge, this is the first example of the use of the NHK in the synthesis of calystegines. This strategy provides a general approach to this class of compound, and should allow the discovery of new lead nortropanes as selective and potent glycosidase inhibitors.

## Experimental

### General method

All reagents were used as received from commercial sources or prepared according the literature. Analytical TLC was performed with 0.20 mm silica gel 60F plates with 254 nm fluorescent indicator. TLC plates were visualized by ultraviolet light or by treatment with a spray of Pancaldi reagent (NH<sub>4</sub>)<sub>6</sub>MoO<sub>4</sub>, Ce(SO<sub>4</sub>)<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O. Column chromatographic purification of products was carried out on silica gel (200–300 mesh). Acidic ion exchange chromatography was performed on Dowex 50WX8–400, H<sup>+</sup> form. Infrared spectra were recorded on an FT-IR spectrometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded with a Bruker AVANCE 500/400/300 spectrometer. The NMR spectra of Cbz-protected compounds were observed at 373K to improve the poor data caused by the rotamers. High resolution mass spectra (HRMS) were recorded on an LTQ/FT linear ion trap mass spectrometer.

**2,3,4-Tri-*O*-benzyl-1-deoxy-1-(benzylamino)-D-xylopyranose (12).** To a solution of 2,3,4-tri-*O*-benzyl-D-xylopyranose **11** (11 g, 26 mmol) in dry DCM (50 mL) with 4 Å mol. Sieves was added *p*TsOH (4.5 g, 26 mmol) followed by benzylamine (27.9 g, 0.26 mol). The mixture was stirred for 4 d at room temperature. The mixture was then filtered and washed with water (3 × 20 mL). The organic layer was dried (Mg<sub>2</sub>SO<sub>4</sub>) and the solvents were removed *in vacuo*. The residue was recrystallized from ethanol to give glycosyl amine **12** (11.6 g, 87%) as a white solid, **12** as a mixture of anomers, Mp: 135–137 °C. [ $\alpha$ ]<sub>D</sub><sup>20</sup> +18.2 (c 1.3 in CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}$ /cm<sup>-1</sup> 3321, 3028, 2866, 1496, 1453, 1352, 1071, 1027, 936, 736, 697; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.34–7.21 (m, 20H), 4.95 (d, *J* = 10.8 Hz, 0.6H), 4.89 (d, *J* = 11.0 Hz, 0.6H), 4.85 (d, *J* = 11.0 Hz, 0.6H), 4.77–4.58 (m, 3.9H), 4.50–4.46 (m, 0.9H), 4.06 (d, *J* = 13.4 Hz, 0.6H), 3.98 (d, *J* = 8.5 Hz, 0.6H), 3.94–3.88 (m, 1.8H), 3.83 (d, *J* = 10.8 Hz, 0.4H), 3.79 (d, *J* = 7.9 Hz, 0.4H), 3.65 (d, *J* = 13.6 Hz, 0.4H), 3.64–3.59 (m, 1.5H), 3.54 (dd, *J* = 8.2, 4.2 Hz, 0.4H), 3.50–3.47 (m, 0.4H), 3.23–3.15 (m, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  140.49, 140.38, 139.09, 138.84, 138.76, 138.62, 138.37, 128.68, 128.62, 128.59, 128.50, 128.39, 128.35, 128.20, 128.16, 128.12, 128.06, 128.02, 127.92, 127.82, 127.20, 90.87, 85.25, 84.26, 82.37, 80.25, 79.42, 78.65, 77.85,

75.86, 75.30, 75.17, 73.37, 73.25, 73.10, 65.19, 60.25, 50.03, 49.71; HRMS-ESI ( $m/z$ ) calcd for  $C_{33}H_{36}NO_4$  [ $M + H$ ]<sup>+</sup> 510.2634, found 510.2630.

**(2R,3R,4S,5R)-5-(Benzylamino)-2,3,4-tris(benzyloxy)hept-6-en-1-ol (13).** To a solution of glycosyl amine **12** (4.8 g, 9.4 mmol) in anhydrous THF (30 mL) was added dropwise a solution of vinylmagnesium bromide in THF (23.5 mL, 1.6 M). The reaction mixture was stirred for 48 h, quenched with a saturated aqueous solution of  $NH_4Cl$ . The aqueous layer was extracted with ethyl acetate (3 × 40 mL). The combined extracts were dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 8/1) to afford **13** (4.5 g, 89%) as a light yellow oil.  $[\alpha]^{20}_D$  -10.8 (c 1.3 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3446, 3063, 3029, 2870, 1496, 1453, 1208, 1088, 1027, 918, 733, 697;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.33–7.22 (m, 20H), 5.78–5.69 (m, 1H), 5.19 (dd,  $J$  = 10.2, 1.3 Hz, 1H), 5.01 (d,  $J$  = 17.2 Hz, 1H), 4.76 (d,  $J$  = 11.3 Hz, 2H), 4.69 (d,  $J$  = 11.4 Hz, 1H), 4.58 (d,  $J$  = 9.4 Hz, 1H), 4.56 (d,  $J$  = 9.9 Hz, 1H), 4.34 (d,  $J$  = 11.7 Hz, 1H), 4.03 (dd,  $J$  = 6.4, 4.6 Hz, 1H), 3.82–3.73 (m, 3H), 3.65 (dd,  $J$  = 11.8, 4.2 Hz, 1H), 3.47–3.42 (m, 2H), 3.07 (dd,  $J$  = 8.3, 3.9 Hz, 1H), 2.07 (s, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  140.43, 138.56, 138.42, 138.20, 138.02, 128.63, 128.38, 128.35, 128.29, 128.27, 128.07, 127.78, 127.71, 127.59, 126.90, 117.41, 82.53, 80.01, 78.43, 74.79, 74.46, 72.09, 61.65, 60.55, 50.43; HRMS-ESI ( $m/z$ ) calcd for  $C_{35}H_{40}NO_4$  [ $M + H$ ]<sup>+</sup> 538.2952, found 538.2940.

**(2R,3R,4S,5R)-5-(N-Benzyl, N-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)hept-6-en-1-ol (14).** To the solution of **13** (537 mg, 1 mmol) in THF (10 mL) was added a drop of water; then  $CbzCl$  (205 mg, 1.2 mmol) and  $NaHCO_3$  (168 mg, 2 mmol) were added and the solution was stirred at room temperature for 12 h. The reaction mixture was quenched with water and extracted with EA (3 × 10 mL). The combined extracts were dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 10/1) to afford **14** (624 mg, 93%) as a colorless oil.  $[\alpha]^{20}_D$  +2.0 (c 1.0 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3463, 3063, 3030, 2927, 1694, 1497, 1454, 1414, 1246, 1108, 1068, 1028, 734, 697;  $^1H$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.33–7.22 (m, 25H), 6.05–5.93 (m, 1H), 5.14 (s, 2H), 5.03 (d,  $J$  = 10.4 Hz, 1H), 4.89 (d,  $J$  = 17.3 Hz, 1H), 4.75–4.40 (m, 9H), 4.25–4.22 (m, 1H), 3.84–3.64 (m, 4H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.39, 138.54, 138.41, 138.20, 138.16, 136.20, 134.59, 127.63, 127.43, 127.32, 127.12, 126.93, 126.83, 126.74, 126.55, 126.09, 117.64, 80.14, 78.53, 78.43, 73.04, 72.77, 71.63, 66.08, 61.28, 60.67, 50.26; HRMS-ESI ( $m/z$ ) calcd for  $C_{43}H_{45}NNaO_6$  [ $M + Na$ ]<sup>+</sup> 694.3145, found 694.3139.

**(3R,4S,5R,6R)-3-(N-Benzyl, N-benzyloxycarbonyl)amino-4,5,6-tris(benzyloxy)-7-(methoxymethoxy)hept-1-ene (15).** To a solution of compound **14** (336 mg, 0.5 mmol) in dry DCM (10 mL) were added DIPEA (129 mg, 1 mmol) and MOMCl (60 mg, 0.75 mmol), and the reaction mixture was stirred for 12 h at room temperature. The reaction was quenched with water and extracted with DCM (3 × 10 mL). The combined organic layers were dried ( $Mg_2SO_4$ ) and concentrated under reduced pressure. Column chromatography purification (silica gel,

petroleum ether/AcOEt 15:1) afforded **15** (322 mg, 90%) as colorless oil.  $[\alpha]^{20}_D$  -3.3 (c 0.6 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3063, 3030, 2926, 2882, 1693, 1496, 1454, 1410, 1208, 1107, 1027, 917, 732, 697;  $^1H$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.30–7.21 (m, 25H), 6.03–5.91 (m, 1H), 5.13 (s, 2H), 5.03 (d,  $J$  = 10.3 Hz, 1H), 4.87 (d,  $J$  = 17.3 Hz, 1H), 4.75–4.38 (m, 11H), 4.22–4.19 (m, 1H), 3.93–3.92 (m, 1H), 3.76 (dd,  $J$  = 11.0, 4.1 Hz, 1H), 3.68–3.63 (m, 2H), 3.26 (s, 3H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.38, 138.23, 138.17, 138.12, 137.99, 136.16, 134.43, 127.63, 127.47, 127.33, 127.15, 126.92, 126.90, 126.77, 126.65, 126.50, 126.13, 117.76, 95.90, 78.31 (2C), 78.05, 72.96, 72.88, 71.71, 67.19, 66.11, 61.31, 54.24, 50.36; HRMS-ESI ( $m/z$ ) calcd for  $C_{45}H_{49}NNaO_7$  [ $M + Na$ ]<sup>+</sup> 738.3401, found 738.3392.

**(3R,4S,5R,6R,Z)-3-(N-Benzyl, N-benzyloxycarbonyl)amino-4,5,6-tris(benzyloxy)-1-iodo-7-(methoxymethoxy)hept-1-ene (16).** Compound **15** (1.06 g, 1.5 mmol) was dissolved in DCM (100 mL) and ozone was passed through the solution for 5 min. Then argon gas was through the solution until the solution turned colourless from blue. DMS (4 equiv.) was added dropwise and the solution was allowed to warm to room temperature and stirred for an additional 10 h. The solvents were removed *in vacuo* and the resulting aldehyde was used directly in the next step without further purification. To a suspension of iodomethyltriphenylphosphonium iodide (1.56 g, 3 mmol) in THF (10 mL) at room temperature was slowly added sodium hexamethyldisilazide (1 M solution in THF, 3 mL). After stirring for 30 min, the dark red solution was cooled to -78 °C and the solution of aldehyde in THF (5 mL) was added slowly. The mixture was allowed to warm to room temperature and, after 30 min, the reaction mixture was quenched with water and extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 10/1) to afford **16** (1.01 g, 80% for 2 steps) as a colorless oil.  $[\alpha]^{20}_D$  +40.0 (c 0.7 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3062, 3030, 2928, 2883, 1697, 1496, 1454, 1411, 1242, 1209, 1110, 1028, 917, 734, 697;  $^1H$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.28–7.22 (m, 25H), 6.62 (s, 2H), 5.12–5.07 (m, 3H), 4.71–4.44 (m, 10H), 4.19 (s, 1H), 3.91 (s, 1H), 3.77–3.69 (m, 3H), 3.27 (s, 3H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.36, 138.28, 138.11, 137.80, 137.15, 135.99, 127.55, 127.40, 127.29, 127.11, 126.88, 126.69, 126.62, 125.96, 95.87, 87.04, 78.21, 77.91, 73.15, 71.83, 71.70, 67.15, 66.29, 61.75, 54.24, 49.34; HRMS-ESI ( $m/z$ ) calcd for  $C_{45}H_{48}NINaO_7$  [ $M + Na$ ]<sup>+</sup> 864.2373, found 864.2367.

**(2R,3R,4S,5R,Z)-5-(N-Benzyl, N-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)-7-iodohept-6-en-1-ol (17).** Compound **16** (430 mg, 0.5 mmol) was dissolved in a solution of HCl (1 N in  $CH_3OH$ ) and stirred under room temperature for 24 h. The solvents were removed *in vacuo* and the residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 8/1) to afford **17** (383 mg, 96%) as a colorless oil.  $[\alpha]^{20}_D$  +36.9 (c 0.65 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3446, 3063, 3030, 2925, 1694, 1496, 1454, 1416, 1243, 1208, 1112, 1028, 734, 697;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.34–7.23 (m, 25H), 6.61 (br, 1H), 6.41 (d,  $J$



= 7.2 Hz, 1H), 5.18 (s, 2H), 4.94–4.32 (m, 10H), 3.70–3.62 (m, 4H), 2.09 (br, 1H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.36, 138.58, 138.37, 137.97, 137.30, 136.05, 127.57, 127.40, 127.30, 127.13, 126.90, 126.82, 126.67, 126.61, 126.51, 125.95, 87.05, 79.98, 78.33, 73.19, 73.09, 71.72, 66.26, 61.70, 60.60, 49.22; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{43}\text{H}_{44}\text{NINaO}_6$  [ $\text{M} + \text{Na}$ ] $^+$  820.2111, found 820.2106.

**(1S,2R,3R,4S,5R)-5-(N-Benzyl, N-benzoyloxycarbonyl)amino-2,3,4-tris(benzoyloxy)cyclohept-6-en-1-ol (18-a) and (1R,2R,3R,4S,5R)-5-(N-Benzyl, N-benzoyloxycarbonyl)amino-2,3,4-tris(benzoyloxy)cyclohept-6-en-1-ol (18-b).** To a solution of oxalyl dichloride (38.1 mg, 0.3 mmol) in DCM (5 mL) was added dropwise DMSO (35.2 mg, 0.45 mmol) at  $-78^\circ\text{C}$ , and the resulting solution was stirred for 20 min. A solution of alcohol **28** (120 mg, 0.15 mmol) in DCM (3 mL) was added dropwise at  $-78^\circ\text{C}$ , and the resulting solution was stirred for 20 min. Then triethylamine (76 mg, 0.75 mmol) was added, and the resulting solution was stirred at  $-78^\circ\text{C}$  for 20 min and at rt for another 30 min. The resulting mixture was diluted with DCM (20 mL) and washed with  $\text{H}_2\text{O}$  ( $3 \times 10$  mL). The organic phase was dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure to afford an aldehyde, which was used directly in the next step. To a solution of the aldehyde in dry DMF (30 mL, freshly distilled from  $\text{CaH}_2$ ) anhydrous  $\text{CrCl}_2$  (183 mg, 1.5 mmol) and  $\text{NiCl}_2$  (2 mg, 0.015 mmol) were added. The resulting mixture was stirred under an atmosphere of Ar at rt for 30 h before being quenched with water (100 mL) and then extracted with ethyl acetate ( $3 \times 30$  mL). The combined organic layers were washed with brine ( $2 \times 30$  mL) before being dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 8/1) to afford two separable diastereomers.

**18-a:** colorless oil (72 mg, 36%),  $[\alpha]_{\text{D}_{20}} +4.4$  (c 1.8 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3445, 3031, 2925, 1698, 1497, 1455, 1250, 1119, 1070, 1027, 736, 697;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.25–7.20 (m, 25H), 5.68–5.58 (m, 2H), 5.14–5.05 (m, 2H), 4.80–4.31 (m, 10H), 4.10–4.05 (m, 1H), 3.74–3.69 (m, 1H), 3.54–3.49 (m, 1H), 3.06 (br, 1H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.59, 138.60, 138.49, 136.44, 132.16, 128.10, 127.83, 127.79, 127.64, 127.31, 127.25, 127.18, 126.95, 126.85, 126.35, 85.40, 83.74, 79.80, 73.48, 73.23, 73.01, 68.10, 66.25, 59.37, 50.78; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{43}\text{H}_{43}\text{NNaO}_6$  [ $\text{M} + \text{Na}$ ] $^+$  692.2983, found 692.2974.

**18-b:** colorless oil (84 mg, 42%),  $[\alpha]_{\text{D}_{20}} -58.0$  (c 1.0 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3445, 3031, 2925, 1698, 1497, 1455, 1250, 1119, 1070, 1027, 736, 697;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.31–7.24 (m, 25H), 5.51–5.42 (m, 2H), 5.13–5.03 (m, 2H), 4.88 (s, 1H), 4.74–4.46 (m, 9H), 4.31–4.25 (m, 1H), 3.96–3.91 (m, 1H), 3.79–3.77 (m, 1H), 3.01 (br, 1H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.72, 138.43, 138.17, 137.89, 136.32, 130.37, 129.18, 127.68, 127.50, 127.14, 126.93, 126.78, 126.61, 126.30, 83.71, 82.05, 81.69, 73.52, 72.14, 71.77, 67.13, 66.02, 58.14, 51.37; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{43}\text{H}_{43}\text{NNaO}_6$  [ $\text{M} + \text{Na}$ ] $^+$  692.2983, found 692.2977.

**(1S,2R,3R,4S,5R)-5-(Benzylamino)-2,3,4-tris(benzoyloxy)cycloheptanol (19).** Pd/C (10%, 10 mg) was

added to a solution of alcohol **18-a** (20 mg, 0.03 mmol) in  $\text{CH}_3\text{OH}$  (5 mL), then the solution was stirred under an atmosphere of  $\text{H}_2$  for 1 h. The Pd/C was filtered off and the solution was concentrated *in vacuo*. Column chromatography purification (silica gel, petroleum ether/AcOEt 1:1) afforded **19** (8.4 mg, 52%) as colourless oil.  $[\alpha]_{\text{D}_{20}} -28.2$  (c 0.4 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3027, 2924, 2855, 1603, 1496, 1454, 1398, 1092, 1069, 735, 696;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.32–7.23 (m, 20H), 4.89 (d,  $J = 11.2$  Hz, 1H), 4.71 (d,  $J = 11.4$  Hz, 1H), 4.69 (d,  $J = 11.3$  Hz, 1H), 4.66 (d,  $J = 11.5$  Hz, 1H), 4.61 (d,  $J = 11.3$  Hz, 1H), 4.51 (d,  $J = 11.5$  Hz, 1H), 3.85–3.81 (m, 2H), 3.78–3.73 (m, 2H), 3.68–3.63 (m, 2H), 3.05–3.03 (m, 1H), 1.91–1.79 (m, 3H), 1.75–1.69 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  138.45, 138.33, 128.68, 128.58, 128.54, 128.20, 128.04, 127.97, 127.91, 127.88, 127.84, 127.06, 85.22, 85.12, 82.64, 75.17, 74.08, 73.26, 70.70, 58.20, 51.62, 28.42, 23.20; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{35}\text{H}_{40}\text{NO}_4$  [ $\text{M} + \text{H}$ ] $^+$  538.2952, found 538.2950.

**(1S,2R,3R,4S,5R)-5-(Benzylamino)cycloheptane-1,2,3,4-tetraol (20).** Pd/C (10%, 5 mg) and concentrated HCl (2 mL) were added to a solution of alcohol **19** (11 mg, 0.02 mmol) in  $\text{CH}_3\text{OH}$  (5 mL), then the solution was stirred under an atmosphere of  $\text{H}_2$  for 1 d. The Pd/C was filtered off and the solution was concentrated *in vacuo*. The residue was dissolved in MeOH and neutralized with aqueous ammonium solution, concentrated *in vacuo*. The above procedure was repeated for three times to ensure complete neutralization. The residue was then purified by an acidic ion exchanger column (Dowex 5W $\times$ 8-400,  $\text{H}^+$  form, Aldrich, column size: 1.3 $\times$ 14 cm), eluting with distilled water (50 mL) and then 1N  $\text{NH}_4\text{OH}$  (50 mL), affording the title compound **19** as a colorless oil (4.7 mg, 85%).  $[\alpha]_{\text{D}_{20}} +8.4$  (c 0.2 in  $\text{CH}_3\text{OH}$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3350, 2929, 1594, 1457, 1418, 1122, 1042, 752, 700;  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.50 (s, 5H), 4.40 (d,  $J = 13.2$  Hz, 1H), 4.27 (d,  $J = 13.2$  Hz, 1H), 3.72 (t,  $J = 9.4$  Hz, 1H), 3.69–3.65 (m, 1H), 3.44 (dd,  $J = 9.0, 7.8$  Hz, 1H), 3.38 (dd,  $J = 9.1, 8.9$  Hz, 1H), 3.18 (td,  $J = 9.3, 5.1$  Hz, 1H), 2.10–2.05 (m, 1H), 1.98–1.91 (m, 1H), 1.87–1.83 (m, 2H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ )  $\delta$  130.48, 129.78, 129.68, 129.33, 76.39, 72.74, 71.98, 71.23, 59.75, 47.88, 27.15, 20.08; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{14}\text{H}_{22}\text{NO}_5$  [ $\text{M} + \text{H}$ ] $^+$  268.1549, found 268.1545.

**(1R,2R,3R,4S,5R)-5-(Benzylamino)-2,3,4-tris(benzoyloxy)cycloheptanol (21).** Pd/C (10%, 10 mg) was added to a solution of alcohol **18-b** (20 mg, 0.03 mmol) in  $\text{CH}_3\text{OH}$  (5 mL), then the solution was stirred under an atmosphere of  $\text{H}_2$  for 1 h. The Pd/C was filtered off and the solution was concentrated *in vacuo*. Column chromatography purification (silica gel, petroleum ether/AcOEt 1:1) afforded **21** (9.8 mg, 61%) as colorless oil.  $[\alpha]_{\text{D}_{20}} -35.0$  (c 0.5 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3028, 2924, 2855, 1603, 1496, 1454, 1398, 1093, 1069, 735, 697;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.32–7.21 (m, 20H), 4.72 (d,  $J = 11.5$  Hz, 1H), 4.63–4.60 (m, 3H), 4.56 (d,  $J = 11.5$  Hz, 1H), 4.44 (d,  $J = 11.5$  Hz, 1H), 4.07 (d,  $J = 7.4$  Hz, 1H), 3.99 (dd,  $J = 6.6, 1.4$  Hz, 1H), 3.95 (dd,  $J = 6.5, 2.8$  Hz, 1H), 3.81 (d,  $J = 13.1$  Hz, 1H), 3.66 (d,  $J = 13.1$  Hz, 1H), 3.57 (dd,  $J = 8.0, 2.6$  Hz, 1H), 3.11 (t,  $J = 7.8$  Hz, 1H), 1.95–1.85 (m, 2H), 1.77–1.71 (m, 1H), 1.56–1.50 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  138.60, 138.32, 137.78, 128.64, 128.57, 128.52, 128.47, 128.19, 128.05, 128.03, 127.97, 127.89, 127.72, 127.00, 84.29,

83.60, 81.11, 73.20, 73.04, 72.97, 70.64, 59.11, 51.50, 29.53, 23.45; HRMS-ESI ( $m/z$ ) calcd for  $C_{35}H_{40}NO_4$  [ $M + H$ ]<sup>+</sup> 538.2952, found 538.2951.

**(2S,3R,4S,5R)-5-(*N*-Benzyl, *N*-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)cyclohept-6-enone (8).**  $NaHCO_3$  (18.5 mg, 0.22 mmol) was added to a well stirred suspension of DMP (63.6 mg, 0.15 mmol) in DCM (5 mL). After 5 min, alcohol **18** (49 mg, 0.07 mmol) was added and the mixture was stirred under room temperature for 2 h. The reaction was quenched with saturated solution of  $Na_2S_2O_3$  and extracted with DCM (3 × 10 mL). The combined organic layers were dried ( $Mg_2SO_4$ ) and concentrated under reduced pressure. Column chromatography purification (silica gel, petroleum ether/AcOEt 10:1) afforded **8** (45 mg, 91%) as colorless oil.  $[\alpha]_D^{20}$  -102.0 (c 1.0 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3063, 3031, 2926, 2869, 1698, 1496, 1429, 1236, 1208, 1096, 1028, 736, 697;  $^1H$  NMR (300 MHz,  $DMSO-d_6$ , 373K)  $\delta$  7.29–7.23 (m, 25H), 6.60 (d,  $J$  = 12.3 Hz, 1H), 5.81 (d,  $J$  = 12.2 Hz, 1H), 5.13 (s, 2H), 5.09–5.06 (m, 1H), 4.81 (d,  $J$  = 15.8 Hz, 1H), 4.65–4.42 (m, 6H), 4.25–4.11 (m, 4H);  $^{13}C$  NMR (75 MHz,  $DMSO-d_6$ , 373K)  $\delta$  197.12, 155.07, 147.68, 137.73, 137.32, 137.19, 136.64, 135.97, 127.75, 127.63, 127.58, 127.48, 127.18, 127.09, 127.03, 126.97, 126.90, 126.85, 126.73, 126.49, 126.08, 83.21, 82.27, 80.33, 72.58, 71.64, 71.15, 66.22, 58.94, 52.20; HRMS-ESI ( $m/z$ ) calcd for  $C_{43}H_{41}NNaO_6$  [ $M + Na$ ]<sup>+</sup> 690.2832, found 690.2826.

**(+)-Calystegine B<sub>2</sub> (3).** Pd/C (10%, 20 mg) was added to a solution of unsaturated ketone **8** (90 mg, 0.07 mmol) in  $CH_3COOH$  (5 mL). After the reaction was stirred under an atmosphere of  $H_2$  for 2 d, TLC (MeOH/ $H_2O$ : 95/5) revealed the formation of a polar compound. The system was flushed with Ar, and the Pd/C was filtered off. After the solution was concentrated *in vacuo*, the residue was dissolved in MeOH and neutralized with aqueous ammonia solution, concentrated *in vacuo*. The above procedure was repeated for three times to ensure complete neutralization. The residue was then purified by an acidic ion exchanger column (Dowex 5W×8-400,  $H^+$  form, Aldrich, column size: 1.3×14cm), eluting with distilled water (50 mL) and then 1N  $NH_4OH$  (50 mL), affording the title compound **3** as a colorless glass (11 mg, 81%).  $[\alpha]_D^{20}$  +28.0 (c 0.5 in  $H_2O$ );  $\nu_{max}/cm^{-1}$  3343, 2927, 1404, 1072, 1015, 952, 823;  $^1H$  NMR (500 MHz,  $D_2O$ )  $\delta$  3.57 (dd,  $J$  = 8.5, 3.6 Hz, 1H), 3.42 (dd,  $J$  = 8.6, 1.6 Hz, 1H), 3.34 (t,  $J$  = 8.6, 1H), 3.31 (dd,  $J$  = 6.7, 4.1 Hz, 1H), 2.03–1.91 (m, 2H), 1.79–1.73 (m, 1H), 1.57–1.51 (m, 1H);  $^{13}C$  NMR (100 MHz,  $D_2O$ )  $\delta$  93.16, 80.41, 77.66, 77.61, 58.58, 31.50, 24.45; HRMS-ESI ( $m/z$ ) calcd for  $C_7H_{14}NO_4$  [ $M + H$ ]<sup>+</sup> 176.0917, found 176.0920. All the data are in accordance with literature values<sup>2d</sup>.

**2,3,4-Tri-*O*-benzyl-1-deoxy-1-(benzylamino)-L-arabinopyranose (23).** To a solution of 2,3,4-tri-*O*-benzyl-L-arabinopyranose (11 g, 26 mmol) in dry DCM (50 mL) with 4 Å mol. sieves was added  $pTsOH$  (4.5 g, 26 mmol) followed by benzylamine (27.9 g, 0.26 mol). The mixture was stirred for 4 d at room temperature. The mixture was then filtered and washed with water (3 × 20 mL). The organic layer was dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. Column chromatography purification (silica gel, petroleum

ether/AcOEt 10:1) afforded **23** as yellow oil (9.26 g, 70%). **23** is a mixture of anomers.  $[\alpha]_D^{20}$  +32.0 (c 1.0 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3321, 3028, 2866, 1496, 1453, 1352, 1071, 1027, 936, 736, 697;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  7.38–7.18 (m, 20H), 4.86 (d,  $J$  = 11.2 Hz, 0.4H), 4.73–4.62 (m, 2.4H), 4.56 (d,  $J$  = 12.5 Hz, 0.7H), 4.52 (d,  $J$  = 6.4 Hz, 1H), 4.49–4.47 (m, 2H), 4.13 (d,  $J$  = 5.9 Hz, 0.4H), 4.06 (d,  $J$  = 13.5 Hz, 0.4H), 4.03 (d,  $J$  = 13.4 Hz, 0.6H), 3.95 (dd,  $J$  = 12.3, 4.9 Hz, 0.4H), 3.87–3.67 (m, 4.9H), 3.62 (dd,  $J$  = 7.4, 2.8 Hz, 0.4H), 3.37 (d,  $J$  = 12.1 Hz, 0.4H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  140.44, 140.31, 138.66, 138.64, 138.49, 138.42, 138.36, 138.14, 128.55, 128.51, 128.49, 128.45, 128.44, 128.41, 128.28, 128.22, 128.20, 128.15, 128.02, 127.89, 127.86, 127.79, 127.74, 126.95, 126.93, 88.85, 84.47, 79.45, 78.61, 77.77, 74.44, 74.00, 73.55, 73.25, 72.92, 72.71, 72.67, 71.45 (2C), 61.93, 61.73, 49.52, 49.38; HRMS-ESI ( $m/z$ ) calcd for  $C_{33}H_{36}NO_4$  [ $M + H$ ]<sup>+</sup> 510.2639, found 510.2635.

**(2S,3R,4S,5R)-5-(Benzylamino)-2,3,4-tris(benzyloxy)hept-6-en-1-ol (24).** To a solution of glycosyl amine **23** (4.6 g, 9.0 mmol) in anhydrous THF (30 mL) was added dropwise a solution of vinylmagnesium bromide in THF (22.5 mL, 1.6 M). The reaction mixture was stirred for 48h, quenched with a saturated aqueous solution of  $NH_4Cl$ . The aqueous layer was extracted with ethyl acetate (3 × 40 mL). The combined extracts were dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 8/1) to afford **24** (4.1 g, 85%) as a light yellow oil.  $[\alpha]_D^{20}$  -11.4 (c 0.7 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3446, 3029, 2922, 1586, 1496, 1453, 1208, 1066, 1027, 925, 733, 697;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.33–7.25 (m, 20H), 5.84–5.75 (m, 1H), 5.26 (d,  $J$  = 10.2 Hz, 1H), 5.13 (d,  $J$  = 17.3 Hz, 1H), 4.78–4.74 (m, 2H), 4.70 (d,  $J$  = 11.2 Hz, 1H), 4.60–4.54 (m, 3H), 4.13 (dd,  $J$  = 5.5, 4.8 Hz, 1H), 3.84–3.78 (m, 3H), 3.70–3.65 (m, 2H), 3.52 (d,  $J$  = 13.2 Hz, 1H), 3.30–3.27 (m, 1H), 2.39–2.29 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  140.06, 138.64, 138.57, 138.28, 138.01, 128.47, 128.41, 128.37, 128.35, 127.90, 127.82, 127.74, 127.71, 127.63, 127.57, 127.01, 118.36, 82.70, 79.81 (2C), 74.94, 74.50, 71.66, 61.81, 61.12, 50.63; HRMS-ESI ( $m/z$ ) calcd for  $C_{35}H_{40}NO_4$  [ $M + H$ ]<sup>+</sup> 538.2952, found 538.2952.

**(2S,3R,4S,5R)-5-(*N*-Benzyl, *N*-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)hept-6-en-1-ol (25).** To the solution of **24** (1.3 g, 2.4 mmol) in THF (10 mL) was added a drop of water, then  $CbzCl$  (412  $\mu$ L, 2.9 mmol) and  $NaHCO_3$  (384 mg, 4.8 mmol) were added and the solution was stirred at room temperature for 12h. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 10/1) to afford **25** (1.45 g, 90%) as a colorless oil.  $[\alpha]_D^{20}$  -2.0 (c 1.0 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3446, 3063, 3031, 2925, 1694, 1497, 1454, 1415, 1245, 1099, 1068, 1028, 734, 697;  $^1H$  NMR (300 MHz,  $DMSO$ , 373K)  $\delta$  7.35–7.20 (m, 25H), 6.06–5.94 (m, 1H), 5.11 (s, 2H), 5.06 (d,  $J$  = 10.3 Hz, 1H), 4.91 (d,  $J$  = 17.4 Hz, 1H), 4.74–4.29 (m, 10H), 3.94 (d,  $J$  = 11.2 Hz, 1H), 3.77 (s, 2H), 3.68 (d,  $J$  = 11.3 Hz, 1H);  $^{13}C$  NMR (75 MHz,  $DMSO$ , 373K)  $\delta$  155.33, 138.49, 138.34, 138.29, 138.14, 136.21, 134.72, 127.59, 127.45, 127.39, 127.31,



127.20, 127.07, 126.78, 126.68, 126.53, 126.12, 118.00, 79.84, 78.56, 78.07, 73.12, 72.45, 70.53, 66.00, 61.54, 59.69, 50.54; HRMS-ESI ( $m/z$ ) calcd for  $C_{43}H_{45}NNaO_6$  [ $M + Na$ ] $^+$  694.3145, found 694.3139.

**(3R,4S,5R,6S)-3-(N-Benzyl, N-benzyloxycarbonyl)amino-4,5,6-tris(benzyloxy)-7-(methoxymethoxy)hept-1-ene (26).** To a solution of amine **25** (3.96 g, 5.9 mmol) in dry DCM (20 mL) were added DIPEA (1.53 g, 11.8 mmol) and MOMCl (713 mg, 8.9 mmol), and the reaction mixture was stirred for 12 h at room temperature. The reaction was quenched with water and extracted with DCM (3  $\times$  10 mL). The combined organic layers were dried ( $Mg_2SO_4$ ) and concentrated under reduced pressure. Column chromatography purification (silica gel, petroleum ether/AcOEt 15:1) afforded **26** (3.9 g, 92%) as colourless oil.  $[\alpha]_D^{20}$  -2.0 (c 0.7 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3063, 3030, 2927, 2883, 1694, 1496, 1454, 1416, 1224, 1107, 917, 733, 696;  $^1H$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.34–7.20 (m, 25H), 6.07–5.95 (m, 1H), 5.12 (s, 2H), 5.07 (d,  $J$  = 10.3 Hz, 1H), 4.92 (d,  $J$  = 17.3 Hz, 1H), 4.75–4.36 (m, 11H), 4.29–4.26 (m, 1H), 3.96–3.92 (m, 2H), 3.76–3.70 (m, 2H), 3.27 (s, 3H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.34, 138.23, 138.15, 136.17, 134.57, 127.60, 127.49, 127.41, 127.32, 127.22, 127.10, 126.76, 126.66, 126.64, 126.30, 126.14, 118.06, 95.97, 78.76, 78.45, 78.40, 73.27, 72.54, 70.76, 66.54, 66.05, 61.49, 54.28, 50.49; HRMS-ESI ( $m/z$ ) calcd for  $C_{45}H_{49}NNaO_7$  [ $M + Na$ ] $^+$  738.3407, found 738.3401.

**(3R,4S,5R,6S,Z)-3-(N-Benzyl, N-benzyloxycarbonyl)amino-4,5,6-tris(benzyloxy)-1-iodo-7-(methoxymethoxy)hept-1-ene(27).** **26** (717 mg, 1 mmol) was dissolved in DCM (70 mL) and ozone was passed through the solution for 5 min. Then argon gas was flushed through the reaction mixture until the solution turned colourless from blue. DMS (4 equiv.) was added dropwise and the solution was allowed to warm to room temperature and stirred for an additional 10 h. The solvents were removed *in vacuo* and the aldehyde was used in the next step without further purification. To a suspension of iodomethyltriphenylphosphonium iodide (1.06 g, 2 mmol) in THF (20 mL) at room temperature was slowly added sodium hexamethyldisilazide (1 M solution in THF, 2 mL). After stirring for 30 min, the dark red solution was cooled to -78  $^{\circ}C$  and the solution of aldehyde in THF (5 mL) was added slowly. The mixture was allowed to warm up to room temperature and, after 30 min, the reaction mixture was quenched with water and extracted with ethyl acetate (3  $\times$  10 mL). The combined extracts were dried ( $Mg_2SO_4$ ) and the solvents were removed *in vacuo*. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 10/1) to afford **27** (631 mg, 75% for 2 steps) as a colorless oil.  $[\alpha]_D^{20}$  +32.0 (c 0.5 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3062, 3030, 2924, 1694, 1621, 1496, 1454, 1411, 1242, 1209, 1111, 1045, 917, 733, 696;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.31–7.17 (m, 25H), 6.65 (br, 1H), 6.41 (d,  $J$  = 7.2 Hz, 1H), 5.16–4.89 (m, 3H), 4.61–4.38 (m, 11H), 3.96–3.87 (m, 2H), 3.75–3.69 (m, 2H), 3.32 (s, 3H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.41, 138.30, 138.21, 138.13, 137.95, 137.27, 136.00, 127.52, 127.48, 127.40, 127.28, 127.05, 126.79, 126.61, 125.99, 95.96, 87.43, 78.59, 78.33, 73.46, 72.71, 70.87,

66.56, 66.24, 61.96, 54.30, 49.32; HRMS-ESI ( $m/z$ ) calcd for  $C_{45}H_{48}NNaO_7$  [ $M + Na$ ] $^+$  864.2373, found 864.2368.

**(2S,3R,4S,5R,Z)-5-(N-Benzyl, N-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)-7-iodohept-6-en-1-ol (28).** **27** (589 mg, 0.7 mmol) was dissolved in a solution of HCl (1 N in  $CH_3OH$ ) and stirred at room temperature for 24h. The solvents were removed *in vacuo* and the residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 8/1) to afford **28** (536 mg, 96%) as a colorless oil.  $[\alpha]_D^{20}$  +36.0 (c 1.0 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3481, 3063, 3031, 2925, 1694, 1496, 1454, 1415, 1246, 1112, 1070, 734, 697;  $^1H$  NMR (300 MHz, DMSO- $d_6$ , 373K)  $\delta$  7.35–7.16 (m, 25H), 6.67–6.58 (m, 2H), 5.08–5.01 (m, 3H), 4.74–4.52 (m, 7H), 4.42 (d,  $J$  = 11.4 Hz, 1H), 4.25–4.23 (m, 1H), 3.91 (d,  $J$  = 11.0 Hz, 1H), 3.75–3.66 (m, 3H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.37, 138.45, 138.31, 138.10, 137.41, 136.04, 127.52, 127.42, 127.36, 127.26, 127.01, 126.84, 126.75, 126.63, 126.52, 125.95, 87.60, 80.02, 78.02, 73.22, 72.53, 70.66, 66.15, 62.10, 59.80, 49.43; HRMS-ESI ( $m/z$ ) calcd for  $C_{43}H_{44}NNaO_6$  [ $M + Na$ ] $^+$  820.2111, found 820.2105.

**(1R,2S,3R,4S,5R)-5-(N-Benzyl, N-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)cyclohept-6-en-1-ol (29).** To a solution of oxalyl dichloride (102 mg, 0.8 mmol) in DCM (5 mL) was added dropwise DMSO (94.8 mg, 1.2 mmol) at -78  $^{\circ}C$ , and the resulting solution was stirred for 20 min. A solution of alcohol **28** (314 mg, 0.4 mmol) in DCM (3 mL) was added dropwise at -78  $^{\circ}C$ , and the resulting solution was stirred for 20 min. Then triethylamine (202 mg, 2 mmol) was added, and the resulting solution was stirred at -78  $^{\circ}C$  for 20 min and at rt for another 30 min. The resulting mixture was diluted with DCM (20 mL) and washed with  $H_2O$  (3  $\times$  10 mL). The organic phase was dried ( $MgSO_4$ ) and concentrated under reduced pressure to afford aldehyde, which was used directly in the next step. To a solution of the aldehyde in dry DMF (30 mL, freshly distilled from  $CaH_2$ ) anhydrous  $CrCl_2$  (487.2 mg, 4 mmol) and  $NiCl_2$  (2.5 mg, 0.02 mmol) were added. The resulting mixture was stirred under an atmosphere of Ar at rt for 30 h before being quenched with water (100 mL) and then extracted with ethyl acetate (3  $\times$  30 mL). The combined organic layers were washed with brine (2  $\times$  30 mL) before being dried ( $MgSO_4$ ) and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 6/1) to afford **29** (187 mg, 70%) as a colorless oil.  $[\alpha]_D^{20}$  -67.6 (c 1.5 in  $CH_2Cl_2$ );  $\nu_{max}/cm^{-1}$  3446, 3063, 3030, 2925, 1698, 1496, 1456, 1403, 1254, 1208, 1101, 1070, 736, 697;  $^1H$  NMR (400 MHz, DMSO- $d_6$ , 343K)  $\delta$  7.38–7.19 (m, 25H), 5.0 (d,  $J$  = 12.3 Hz, 1H), 5.42 (dd,  $J$  = 12.3, 3.1 Hz, 1H), 5.06–5.04 (m, 2H), 4.86 (d,  $J$  = 6.5 Hz, 1H), 4.73–4.52 (m, 6H), 4.30–4.23 (m, 3H), 4.06–3.99 (m, 2H), 3.87 (d,  $J$  = 7.6 Hz, 1H);  $^{13}C$  NMR (75 MHz, DMSO- $d_6$ , 373K)  $\delta$  155.18, 138.53, 138.33, 137.95, 136.21, 130.69, 127.55, 127.44, 126.91, 126.82, 126.75, 126.60, 126.53, 126.26, 126.11, 81.17, 80.20, 78.87, 71.94, 71.79, 71.66, 68.09, 65.90, 59.94, 50.38; HRMS-ESI ( $m/z$ ) calcd for  $C_{43}H_{43}NNaO_6$  [ $M + Na$ ] $^+$  692.2983, found 692.2972.

**(2R,3R,4S,5R)-5-(N-Benzyl, N-benzyloxycarbonyl)amino-2,3,4-tris(benzyloxy)cyclohept-6-enone (30).**  $NaHCO_3$  (53 mg, 0.63

mmol) was added to a well stirred suspension of DMP (178 mg, 0.42 mmol) in DCM (5 mL). After 5 min, alcohol **29** (140 mg, 0.21 mmol) was added and the mixture was stirred under room temperature for 2 h. The reaction was quenched with saturated solution of  $\text{Na}_2\text{S}_2\text{O}_3$  and extracted with DCM (3  $\times$  10 mL). The combined organic layers were dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. Column chromatography purification (silica gel, petroleum ether/AcOEt 10:1) afforded **30** (191 mg, 93%) as colourless oil.  $[\alpha]_{\text{D}}^{20}$  -42.2 (c 0.9 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3063, 3031, 2924, 1689, 1496, 1454, 1236, 1099, 1028, 736, 697;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ , 373K)  $\delta$  7.36–7.27 (m, 25H), 6.51 (d,  $J$  = 12.2 Hz, 1H), 5.95 (d,  $J$  = 12.2 Hz, 1H), 5.10 (s, 2H), 4.83–4.46 (m, 6H), 4.38–4.15 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ , 373K)  $\delta$  194.82, 154.77, 144.44, 137.97, 137.73, 137.57, 137.25, 135.97, 129.36, 129.71, 127.64, 127.53, 127.48, 127.20, 127.02, 126.83, 126.72, 126.44, 84.09, 80.52, 79.89, 71.43 (2C), 71.05, 66.21, 62.26, 52.33; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{43}\text{H}_{41}\text{NNaO}_6$  [ $M + \text{Na}$ ] $^+$  690.2826, found 690.2817.

**(+)-Calystegine B<sub>3</sub> (4).** Pd/C (10%, 20 mg) was added to a solution of unsaturated ketone **30** (67 mg, 0.1 mmol) in  $\text{CH}_3\text{COOH}$  (5 mL). After the reaction was stirred under an atmosphere of  $\text{H}_2$  for 2 d, TLC (AcOEt/MeOH: 5/1) revealed the formation of a polar compound. The system was bubbled with  $\text{Ar}_2$ , and the Pd/C was filtered off. After the solution was concentrated *in vacuo*, the residue was purified with acidic ion exchanger column (Dowex 5W $\times$ 8-400,  $\text{H}^+$  form, Aldrich, column size: 1.3 $\times$ 14cm), eluting with distilled water (50 mL) and then 1N  $\text{NH}_4\text{OH}$  (50 mL), an basic ion exchange column (Amberlite IRA-400,  $\text{OH}^-$  form, Alfa, column size: 1.3 $\times$ 14cm), affording calystegine B<sub>3</sub> **4** as a white powder (14 mg, 80%).  $[\alpha]_{\text{D}}^{20}$  +68.0 (c 0.5 in  $\text{H}_2\text{O}$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3343, 2927, 1404, 1072, 1015, 952, 823;  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ )  $\delta$  3.83 (d,  $J$  = 3.9 Hz, 1H), 3.65 (dd,  $J$  = 9.5, 3.6 Hz, 1H), 3.61 (dd,  $J$  = 9.4, 3.9 Hz, 1H), 3.28 (dd,  $J$  = 6.8, 3.7 Hz, 1H), 1.96–1.88 (m, 1H), 1.81–1.72 (m, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ )  $\delta$  93.13, 77.20, 75.14, 72.99, 58.28, 34.04, 22.95; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_7\text{H}_{14}\text{NO}_4$  [ $M + \text{H}$ ] $^+$  176.0917, found 176.0917. All the data are in accordance with literature values<sup>2d</sup>.

**(1R,2S,3R,4S,5R)-5-(Benzylamino)-2,3,4-tris(benzyloxy)cycloheptanol (31).** Pd/C (10%, 10 mg) was added to a solution of alcohol **29** (20 mg, 0.03mmol) in  $\text{CH}_3\text{OH}$  (5 mL), then the solution was stirred under an atmosphere of  $\text{H}_2$  for 1h. The Pd/C was filtered off and the solution was concentrated *in vacuo*. Column chromatography purification (silica gel, petroleum ether/AcOEt1:1) afforded **31** (11.3 mg, 70%) as colorless oil.  $[\alpha]_{\text{D}}^{20}$  -34.3 (c 0.3 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3062, 3029, 2924, 2855, 1604, 1496, 1454, 1397, 1093, 1071, 1028, 735, 697;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33–7.22 (m, 20H), 4.72 (d,  $J$  = 12.0 Hz, 1H), 4.63 (d,  $J$  = 12.0 Hz, 1H), 4.50 (d,  $J$  = 11.5 Hz, 1H), 4.46 (d,  $J$  = 11.8 Hz, 1H), 4.43–4.38 (m, 2H), 4.11 (td,  $J$  = 9.1, 5.8 Hz, 1H), 4.01 (d,  $J$  = 3.1 Hz, 1H), 3.81 (d,  $J$  = 13.0 Hz, 1H), 3.66 (d,  $J$  = 13.1 Hz, 1H), 3.63 (br, 1H), 3.52 (d,  $J$  = 9.1 Hz, 1H), 2.72 (s, 1H), 2.21–2.15 (m, 1H), 1.79–1.78 (m, 2H), 1.45–1.40 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  138.27, 138.00, 137.89, 128.57, 128.42, 128.40, 128.13, 127.95, 127.93, 127.91, 127.76, 127.72, 126.93, 83.44, 81.85, 77.67, 72.84, 72.30,

71.89, 69.80, 62.30, 51.17, 30.88, 23.28; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{35}\text{H}_{40}\text{NO}_4$  [ $M + \text{H}$ ] $^+$  538.2952, found 538.2954.

**(1S,2S,3R,4S,5R)-8-Benzyl-2,3,4-tris(benzyloxy)-8-azabicyclo[3.2.1]octane (32).** Pd/C (10%, 20 mg) was added to a solution of unsaturated ketone **29** (67 mg, 0.1 mmol) in  $\text{CH}_3\text{OH}$  (5 mL). After the reaction was stirred under an atmosphere of  $\text{H}_2$  for 5 h, the Pd/C was filtered off and the solution was concentrated under reduced pressure. To the solution of the residue in  $\text{CH}_2\text{Cl}_2$  (5ml) ( $\text{Boc}$ )<sub>2</sub>O (32.8 mg, 0.15 mmol) and  $\text{Et}_3\text{N}$  (21 mg, 0.2 mmol) were added. The reaction mixture was stirred at rt for 2 h.  $\text{CH}_2\text{Cl}_2$  (20 mL) was added, and the solution was washed with  $\text{H}_2\text{O}$  (2  $\times$  10 mL). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. Methanesulfonyl chloride (17.2 mg, 0.15mmol) and  $\text{Et}_3\text{N}$  (21 mg, 0.2 mmol) were added to a solution of the residue in  $\text{CH}_2\text{Cl}_2$  (5 ml). The reaction mixture was stirred at rt for 2 h.  $\text{CH}_2\text{Cl}_2$  (20 mL) was added, and the solution was washed with  $\text{H}_2\text{O}$  (2  $\times$  10 mL). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residue was redissolved in  $\text{CH}_2\text{Cl}_2$  (5 ml) and  $\text{CF}_3\text{COOH}$  (2 ml) was added. After the reaction mixture was stirred at rt for 2 h, the solution was concentrated under reduced pressure. The saturated sodium bicarbonate solution (10 ml) was added and then extracted with ethyl acetate (3  $\times$  10 mL). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/AcOEt = 8/1) to afford **32** (22 mg, 42%) as a colorless oil.  $[\alpha]_{\text{D}}^{20}$  +68.0 (c 0.5 in  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3028, 2921, 2850, 1496, 1453, 1205, 1101, 731, 696;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.46 (d,  $J$  = 7.0 Hz, 2H), 7.35–7.24 (m, 18H), 4.72 (d,  $J$  = 11.8 Hz, 1H), 4.64 (d,  $J$  = 11.9 Hz, 1H), 4.61 (s, 2H), 4.52 (d,  $J$  = 12.6 Hz, 1H), 4.42 (d,  $J$  = 12.6 Hz, 1H), 3.97 (dd,  $J$  = 8.8, 3.7 Hz, 1H), 3.68 (d,  $J$  = 13.5 Hz, 1H), 3.52 (d,  $J$  = 13.5 Hz, 1H), 3.48–3.41 (m, 2H), 3.36–3.33 (m, 2H), 1.95–1.81 (m, 3H), 1.26–1.17 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  140.06, 139.42, 139.16, 139.01, 128.82, 128.37, 128.31, 128.02, 127.71, 127.64, 127.43, 127.40, 127.00, 81.27, 78.11, 76.93, 72.41, 72.05, 71.22, 62.19, 60.18, 56.69, 23.68, 21.28; HRMS-ESI ( $m/z$ ) calcd for  $\text{C}_{35}\text{H}_{38}\text{NO}_3$  [ $M + \text{H}$ ] $^+$  520.2846, found 520.2840.

## Acknowledgements

We thank the National Natural Science Foundation of China (No. 21272240), the Ministry of Science and Technology of P. R. China (No. 2012CB822101, 2011CB808603), National Science and Technology Major Projects for "Major New Drugs Innovation and Development" (2013ZX09508104), and the Chinese Academy of Science for financial support. This work was supported in part by a Grant-in-Aid for Scientific Research (C) (No: 26460143) (AK) from the Japanese Society for the Promotion of Science (JSPS) and a Leverhulme research fellowship (GWJF).

## Notes and references

- 1 D. Tepfer, A. Goldmann, N. Pamboukdjian, M. Maille, A. Lepingle, D. Chevalier, J. Dénarié and C. Rosenberg, *J. Bacteriol.*, 1988, **170**, 1153-1161.
- 2 (a) N. Asano, A. Kato, H. Kizu, K. Matsui, A. A. Watson and R. J. Nash, *Carbohydr. Res.*, 1996, **293**, 195-204; (b) B. Dräger, A. van Almsick and G. Mrachatz, *Planta Med.*, 1995, **61**, 577-579; (c) B. Dräger, *Phytochem. Anal.*, 1995, **6**, 31-37; (d) N. Asano, A. Kato, K. Oseki, H. Kizu and K. Matsui, *Eur. J. Biochem.*, 1995, **229**, 369-376; (e) N. Asano, E. Tomioka, H. Kizu and K. Matsui, *Carbohydr. Res.*, 1994, **253**, 235-245; (f) N. Asano, K. Oseki, E. Tomioka, H. Kizu and K. Matsui, *Carbohydr. Res.*, 1994, **259**, 243-255; (g) R. J. Nash, M. Rothschild, E. A. Porter, A. A. Watson, R. D. Waigh and P. G. Waterman, *Phytochemistry*, 1993, **34**, 1281-1283; (h) A. Goldmann, M.-L. Milat, P.-H. Ducrot, J.-Y. Lallemand, M. Maille, A. Lepingle, I. Charpin and D. Tepfer, *Phytochemistry*, 1990, **29**, 2125-2127; (i) P. H. Ducrot and J. Y. Lallemand, *Tetrahedron Lett.*, 1990, **31**, 3879-3882.
- 3 N. Asano, A. Kato, K. Matsui, A. A. Watson, R. J. Nash, R. J. Molyneux, L. Hackett, J. Topping and B. Winchester, *Glycobiology*, 1997, **7**, 1085-1088.
- 4 For reviews of calystegines, see: (a) M. S. Pino-Gonzalez, N. Ona and A. Romero-Carrasco, *Mini-Rev. Med. Chem.*, 2012, **12**, 1477-1484; (b) S. Biastoff and B. Dräger, in *The Alkaloids: Chemistry and Biology*, ed. A. C. Geoffrey, Academic Press, 2007, vol. 64, pp. 49-102; (c) G. P. Pollini, S. Benetti, C. De Risi and V. Zanirato, *Chem. Rev.*, 2006, **106**, 2434-2454; (d) B. Dräger, *Nat. Pro. Rep.*, 2004, **21**, 211-223.
- 5 (a) N. Asano, A. Kato, M. Miyauchi, H. Kizu, T. Tomimori, K. Matsui, R. J. Nash and R. J. Molyneux, *Eur. J. Biochem.*, 1997, **248**, 296-303; (b) N. Asano, A. Kato, Y. Yokoyama, M. Miyauchi, M. Yamamoto, H. Kizu and K. Matsui, *Carbohydr. Res.*, 1996, **284**, 169-178.
- 6 N. Asano, R. J. Nash, R. J. Molyneux and G. W. J. Fleet, *Tetrahedron: Asymmetry*, 2000, **11**, 1645-1680.
- 7 (a) A. Kato, I. Nakagome, S. Nakagawa, Y. Koike, R. J. Nash, I. Adachi and S. Hirono, *Biorg. Med. Chem.*, 2014, **22**, 2435-2441; (b) A. Kato, L. Wang, K. Ishii, J. Seino, N. Asano and T. Suzuki, *J. Biochem.*, 2011, **149**, 415-422; (c) S. E. Milner, N. P. Brunton, P. W. Jones, N. M. O'Brien, S. G. Collins and A. R. Maguire, *J. Agric. Food Chem.*, 2011, **59**, 3454-3484; (d) R. J. Molyneux, Y. T. Pan, A. Goldmann, D. A. Tepfer and A. D. Elbein, *Arch. Biochem. Biophys.*, 1993, **304**, 81-88.
- 8 (a) J. Y. Sun, M. Z. Zhu, S. W. Wang, S. Miao, Y. H. Xie and J. B. Wang, *Phytomedicine*, 2007, **14**, 353-359; (b) P. E. Goss, M. A. Baker, J. P. Carver and J. W. Dennis, *Clin. Cancer Res.*, 1995, **1**, 935-944.
- 9 P. Greimel, J. Spreitz, A. E. Stütz and T. M. Wrodnigg, *Curr. Top. Med. Chem.*, 2003, **3**, 513-523.
- 10 F. M. Platt, G. R. Neises, G. Reinkensmeier, M. J. Townsend, V. H. Perry, R. L. Proia, B. Winchester, R. A. Dwek and T. D. Butters, *Science*, 1997, **276**, 428-431.
- 11 T. M. Wrodnigg and F. K. Sprenger, *Mini-Rev. Med. Chem.*, 2004, 437-459.
- 12 T. D. Butters, R. A. Dwek and F. M. Platt, *Chem. Rev.*, 2000, **100**, 4683-4696.
- 13 For representative methods for the synthesis of calystegines, see: (a) T. S. Rasmussen and H. H. Jensen, *Carbohydr. Res.*, 2011, **346**, 2855-2861; (b) P. Moosophon, M. C. Baird, S. Kanokmedhakul and S. G. Pyne, *Eur. J. Org. Chem.*, 2010, 3337-3344; (c) R. N. Monrad, C. B. Pipper and R. Madsen, *Eur. J. Org. Chem.*, 2009, 3387-3395; (d) R. Csuk, E. Prell and S. Reissmann, *Tetrahedron*, 2008, **64**, 9417-9422; (e) P. R. Skaanderup and R. Madsen, *J. Org. Chem.*, 2003, **68**, 2115-2122; (f) P. R. Skaanderup and R. Madsen, *Chem. Commun.*, 2001, 1106-1107; (g) F. D. Boyer and I. Hanna, *Tetrahedron Lett.*, 2001, **42**, 1275-1277; (h) F.-D. Boyer and J.-Y. Lallemand, *Synlett*, 1992, 969-971; (i) F.-D. Boyer and J.-Y. Lallemand, *Tetrahedron*, 1994, **50**, 10443-10458; (j) F.-D. Boyer, P.-H. Ducrot, V. Henryon, J. Soulié and J.-Y. Lallemand, *Synlett*, 1992, 357-359; (k) T. Faltg, J. Soulie, J. Y. Lallemand and L. Ricard, *Tetrahedron: Asymmetry*, 1999, **10**, 2165-2174; (l) J. Soulie, T. Faltg, J. F. Betzer and J. Y. Lallemand, *Tetrahedron*, 1996, **52**, 15137-15146; (m) O. Duclos, M. Mondange, A. Duréault and J. C. Depezay, *Tetrahedron Lett.*, 1992, **33**, 8061-8064; (n) J. Marco-Contelles and E. de Opazo, *J. Org. Chem.*, 2002, **67**, 3705-3717; (o) A. Kamimura, K. Miyazaki, S. Suzuki, S. Ishikawa and H. Uno, *Org. Biomol. Chem.*, 2012, **10**, 4362-4366; (p) Y.-L. Chen, H. Redlich, K. Bergander and R. Froehlich, *Org. Biomol. Chem.*, 2007, **5**, 3330-3339; (q) C. R. Johnson and S. J. Bis, *J. Org. Chem.*, 1995, **60**, 615-623.
- 14 For recent synthesis of calystegine related compounds, see: (a) A. H. Viuff, L. M. Besenbacher, A. Kamori, M. T. Jensen, M. Kilian, A. Kato and H. H. Jensen, *Org. Biomol. Chem.*, 2015, **13**, 9637-9658; (b) A. Kato, Z.-L. Zhang, H.-Y. Wang, Y.-M. Jia, C.-Y. Yu, K. Kinami, Y. Hirokami, Y. Tsuji, I. Adachi, R. J. Nash, G. W. J. Fleet, J. Koseki, I. Nakagome and S. Hirono, *J. Org. Chem.*, 2015, **80**, 4501-4515; (c) S. H. Kyne, J. A. L. Miles, J. M. Percy and K. Singh, *J. Org. Chem.*, 2012, **77**, 991-998; (d) I. Delso, T. Tejero, A. Goti and P. Merino, *J. Org. Chem.*, 2011, **76**, 4139-4143; (e) R. Beniazza, V. Desvergnès, G. Mehta, N. Blanchard, F. Robert and Y. Landais, *J. Org. Chem.*, 2011, **76**, 791-799; (f) T. S. Rasmussen and H. H. Jensen, *Org. Biomol. Chem.*, 2010, **8**, 433-441; (g) E. Girard, V. Desvergnès, C. Tarnus and Y. Landais, *Org. Biomol. Chem.*, 2010, **8**, 5628-5634; (h) D. Lo Re, F. Franco, F. Sanchez-Cantalejo and J. A. Tamayo, *Eur. J. Org. Chem.*, 2009, 1984-1993; (i) K. P. Kaliappan, P. Das, S. T. Chavan and S. G. Sabharwal, *J. Org. Chem.*, 2009, **74**, 6266-6274; (j) V. Chagnault, P. Compain, K. Lewinski, K. Ikeda, N. Asano and O. R. Martin, *J. Org. Chem.*, 2009, **74**, 3179-3182; (k) T. K. M. Shing, W. F. Wong, T. Ikeno and T. Yamada, *Org. Lett.*, 2007, **9**, 207-209; (l) B. Groetzl, S. Handa and J. R. Malpass, *Tetrahedron Lett.*, 2006, **47**, 9147-9150; (m) M. I. Garcia-Moreno, C. O. Mellet and J. M. G. Fernandez, *Eur. J. Org. Chem.*, 2004, 1803-1819; (n) S. D. Koulocheri, E. N. Pitsinos and S. A. Haroutounian, *Synthesis*, 2002, 1707-1710; (o) P. A. V. van Hooft, R. E. J. N. Litjens, G. A. van der Marel, C. A. A. van Boeckel and J. H. van Boom, *Org. Lett.*, 2001, **3**, 731-733; (p) M. I. Garcia-Moreno, J. M. Benito, C. O. Mellet and J. M. G. Fernandez, *J. Org. Chem.*, 2001, **66**, 7604-7614; (q) O. Duclos, A. Duréault and J. C. Depezay, *Tetrahedron Lett.*, 1992, **33**, 1059-1062.
- 15 (a) Y.-X. Li, K. Kinami, Y. Hirokami, A. Kato, J.-K. Su, Y.-M. Jia, G. W. J. Fleet and C.-Y. Yu, *Org. Biomol. Chem.*, 2016, **14**, 2249-2263; (b) Y.-X. Li, R. Iwaki, A. Kato, Y.-M. Jia, G. W. J. Fleet, X. Zhao, M. Xiao and C.-Y. Yu, *Eur. J. Org. Chem.*, 2016, 1429-1438; (c) Y.-X. Li, Y. Shimada, K. Sato, A. Kato, W. Zhang, Y.-M. Jia, G. W. J. Fleet, M. Xiao and C.-Y. Yu, *Org. Lett.*, 2015, **17**, 716-719; (d) Y.-X. Li, Y. Shimada, I. Adachi, A. Kato, Y.-M. Jia, G. W. J. Fleet, M. Xiao and C.-Y. Yu, *J. Org. Chem.*, 2015, **80**, 5151-5158; (e) J.-S. Zhu, S. Nakagawa, W. Chen, I. Adachi, Y.-M. Jia, X.-G. Hu, G. W. J. Fleet, F. X. Wilson, T. Nitoda, G. Horne, R. van Well, A. Kato and C.-Y. Yu, *J. Org. Chem.*, 2013, **78**, 10298-10309; (f) W.-B. Zhao, S. Nakagawa, A. Kato, I. Adachi, Y.-M. Jia, X.-G. Hu, G. W. J. Fleet, F. X. Wilson, G. Horne, A. Yoshihara, K. Izumori and C.-Y. Yu, *J. Org. Chem.*, 2013, **78**, 3208-3221; (g) H. Zhao, A. Kato, K. Sato, Y.-M. Jia and C.-Y. Yu, *J. Org. Chem.*, 2013, **78**, 7896-7902; (h) W.-Y. Xu, R. Iwaki, Y.-M. Jia, W. Zhang, A. Kato and C.-Y. Yu, *Org. Biomol.*

- Chem.*, 2013, **11**, 4622-4639; (i) Z.-L. Zhang, S. Nakagawa, A. Kato, Y.-M. Jia, X.-G. Hu and C.-Y. Yu, *Org. Biomol. Chem.*, 2011, **9**, 7713-7719; (j) W. Zhang, K. Sato, A. Kato, Y.-M. Jia, X.-G. Hu, F. X. Wilson, R. van Well, G. Horne, G. W. J. Fleet, R. J. Nash and C.-Y. Yu, *Org. Lett.*, 2011, **13**, 4414-4417; (k) X.-G. Hu, B. Bartholomew, R. J. Nash, F. X. Wilson, G. W. J. Fleet, S. Nakagawa, A. Kato, Y.-M. Jia, R. v. Well and C.-Y. Yu, *Org. Lett.*, 2010, **12**, 2562-2565; (l) C.-Y. Yu and M.-H. Huang, *Org. Lett.*, 2006, **8**, 3021-3024.
- 16 H.-H. Chang, N. Asano, S. Ishii, Y. Ichikawa and J.-Q. Fan, *FEBS J.*, 2006, **273**, 4082-4092.
- 17 For the representative applications of NHK reaction in total synthesis, see: (a) B. Bolte, J. A. Basutto, C. S. Bryan, M. J. Garson, M. G. Banwell and J. S. Ward, *J. Org. Chem.*, 2015, **80**, 460-470; (b) S. S. Palimkar, J. i. Uenishi and H. Ii, *J. Org. Chem.*, 2012, **77**, 388-399; (c) J. C. Killen, L. C. Axford, S. E. Newberry, T. J. Simpson and C. L. Willis, *Org. Lett.*, 2012, **14**, 4194-4197; (d) S. S. Palimkar and J. i. Uenishi, *Org. Lett.*, 2010, **12**, 4160-4163; (e) D. K. Mohapatra, P. P. Das, M. R. Pattanayak, G. Gayatri, G. N. Sastry and J. S. Yadav, *Eur. J. Org. Chem.*, 2010, 4775-4784; (f) J. Pospíšil, C. Müller and A. Fürstner, *Chem. Eur. J.*, 2009, **15**, 5956-5968; (g) T. M. Baker, D. J. Edmonds, D. Hamilton, C. J. O'Brien and D. J. Procter, *Angew. Chem. Int. Ed.*, 2008, **47**, 5631-5633.
- 18 (a) L. C. Dias, L. G. de Oliveira, J. D. Vilcachagua and F. Nigsch, *J. Org. Chem.*, 2005, **70**, 2225-2234; (b) G. Stork and K. Zhao, *Tetrahedron Lett.*, 1989, **30**, 2173-2174; (c) H. J. Bestmann, H. C. Rippel and R. Dostalek, *Tetrahedron Lett.*, 1989, **30**, 5261-5262.
- 19 (a) J. Saha and M. W. Pecuh, *Chem. Eur. J.*, 2011, **17**, 7357-7365; (b) J. Saha and M. W. Pecuh, *Org. Lett.*, 2009, **11**, 4482-4484.
- 20 (a) A. S. Kireev, O. N. Nadein, V. J. Agustin, N. E. Bush, A. Evidente, M. Manpadi, M. A. Ogasawara, S. K. Rastogi, S. Rogelj, S. T. Shors and A. Kornienko, *J. Org. Chem.*, 2006, **71**, 5694-5707; (b) O. N. Nadein and A. Kornienko, *Org. Lett.*, 2004, **6**, 831-834.
- 21 (a) L. Cipolla, M. R. Fernandes, M. Gregori, C. Airolidi and F. Nicotra, *Carbohydr. Res.*, 2007, **342**, 1813-1830; (b) L. Cipolla, L. Lay, F. Nicotra, C. Pangrazio and L. Panza, *Tetrahedron*, 1995, **51**, 4679-4690.
- 22 (a) J. Mulzer, T. Schulze, A. Strecker and W. Denzer, *J. Org. Chem.*, 1988, **53**, 4098-4103; (b) M. D. Lewis and Y. Kishi, *Tetrahedron Lett.*, 1982, **23**, 2343-2346.