ORGANIC LETTERS

2007
Vol. 9, No. 19
3713−3716

Upper Rim Appended Hybrid Calixarenes via Click Chemistry

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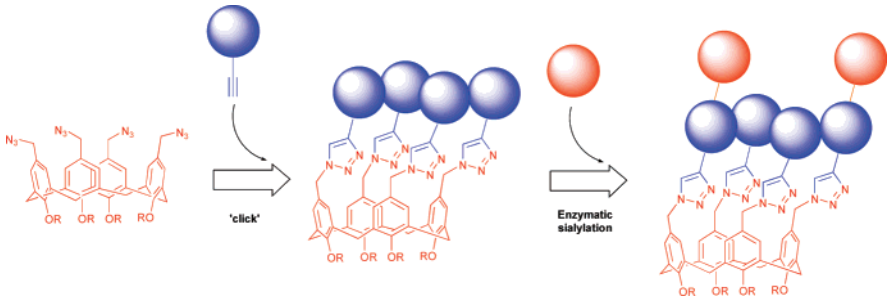
Received May 15, 2007

ABSTRACT

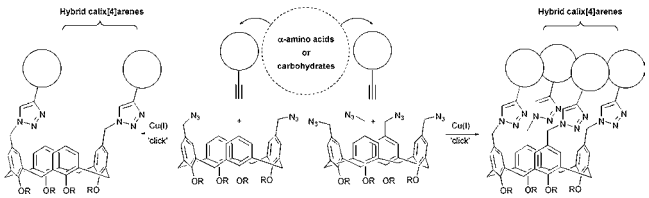


We report the application of "click" chemistry for the synthesis of hybrid calixarenes appended on the upper rim with carbohydrate and *N,C*-protected α-amino acids. The chemoselective *N*- or *C*-deprotection of the α-amino acids and their subsequent transformation into dipeptides is described. The first example of a chemo-enzymatic synthesis on upper rim derived calix[4]arenes using *trans*-sialidase affords sialylated lactose calix[4]arenes. Our innovative chemo-enzymatic process paves the way for further applications.

In an ongoing project, we required a powerful synthetic protocol capable of rapidly and efficiently synthesizing structurally diverse upper-rim appended α-amino acids or carbohydrate-derived calix[4]arenes. In connection with these studies, we wanted to investigate the structural diversification of a carbohydrate calix[4]arene using a chemo-enzymatic process. Appending sialic acids via a chemo-enzymatic procedure generating upper-rim derived sialylated-β-lactoside calixarene hybrids has not, to date, been reported. Initiating this work, we focused on the possibility that "click"[1] chemistry can be employed, for the first time, for the efficient construction of *upper-rim* derived "hybrid" calix[4]arenes (Scheme 1).

The discovery that copper(I) species are able to catalyze the Hüisgen reaction ("click" chemistry) has spawned a myriad of diverse applications.[2] Our desire to employ "click" chemistry for the synthesis of hybrid calixarenes focused on the fact that upper rim azide-derived calix[4]arenes are readily synthesized in multigram quantities using cheap starting materials and in high yields.[3] Using these azides as our starting materials together with readily synthesized alkyne-derived α-amino acids or carbohydrates, the "click" chemistry is quick, atom-efficient, and versatile. As part of our future research, we intend to further manipulate and structurally diversify the appended α-amino acid and carbohydrate groups. For this reason, it was important that the method of attachment be chemically robust. The triazole heterocycle is surprisingly stable. It is not degraded by exposure to dilute acid, base, or low-pressure hydrogenation conditions. Furthermore, although "click" chemistry has been employed for the appendage of water-soluble groups onto the *lower rim* of calix[4]arenes,[4] the chemistry has not been utilized for the construction of *upper rim* appended α-amino

Scheme 1. "Click" Chemistry Derived Upper Rim Hybrid Calix[4]arenes



(1) Hartmuth, K. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(2) See Rodionov, V. O.; Forkin, V. V.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2210 and references therein.

10.1021/ol071047t CCC: $37.00
Published on Web 08/15/2007

acids. There is a dearth of protocols that efficiently afford upper rim appended hybrid calix[4]arenes. Those that are available are few in number,[5] protracted, and inefficient.

Our long-term synthetic strategy for the hybrid calixarenes required a flexible approach to controlling the conformation of the calix[4]arene. Independently, Hamilton et al. and Ungaro et al. have demonstrated that lengthening or shortening *O*-alkyl chains attached to the *lower rim* to be an efficient "conformational tool".[6] Obviously, appending the α-amino acids, their derivatives, or the carbohydrates onto the lower rim would inhibit this future avenue of research.

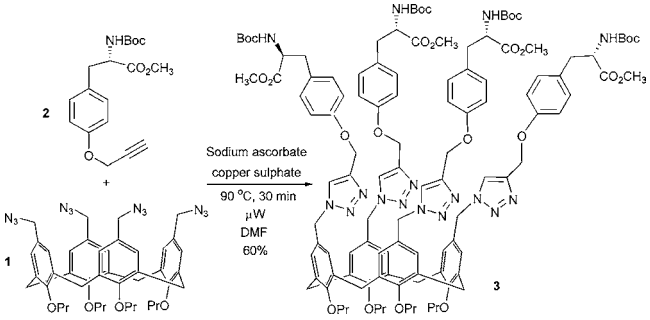
Although the prospective applications of *upper rim* appended hybrid calixarenes are evident, their current lack of ready availability is severely impeding their potential. The methodology presented here surmounts these problems.

With reaction efficiency in mind, preliminary endeavors focused on utilizing the one-pot in situ azide synthesis/"click" protocol reported by Van der Eycken et al.[7] Employing microwave irradiation, *O*-*n*-propoxychloromethylcalix[4]-arene, sodium azide, and *N*-Boc-*O*-propargyl-(*S*)-tyrosine methyl ester, the desired "click" calix[4]arene adducts were not formed. All of the starting materials were returned in good mass balances.

Switching to a stepwise protocol, the synthesis of the core starting material **1** was readily undertaken via an S_N2 displacement (NaN_3, 90 °C, DMF, μW, 90% yield) on *O*-*n*-propoxychloromethylcalix[4]arene. Utilizing a procedure reported by Sharpless et al., a copper(I)-catalyzed [3 + 2]-dipolar cycloaddition reaction between calix[4]arene derivative **1** and (*S*)-tyrosine derivative **2** was attempted.[8] An aqueous *tert*-butyl alcohol (1:1 v/v) solution of **1** and **2** was stirred (96 h) at ambient temperature with copper, copper(II) sulfate, and sodium ascorbate. No reaction was observed, and **1** and **2** were recovered in good yields. Closer inspection of the reaction indicated that **1** was not particularly soluble in the aqueous *tert*-butyl alcohol at ambient temperature. Our premise that conducting the reaction at a higher temperature (100 °C, μW) may help to solubilize the reactants was partially valid; a single regioisomer of **3** was isolated, although in a poor 10% yield. Concerned that the solubility of **1** in aqueous *tert*-butyl alcohol at 100 °C may still be a problem, it was substituted for DMF. Aqueous DMF rectified the solubility problem but to our dismay had no positive effect on the outcome of the reaction when conducted at ambient temperature or when a diverse selection of metal salts were tested, i.e., copper(I) acetate, bis-(triphenylphosphine)copper(I) bromide, copper(I) iodide, copper sulfate/copper turnings. All of these reactions returned **1** and **2** in high yields. Gratifyingly, when the reaction was repeated using our original combination of copper, copper-(II) sulfate, and sodium ascorbate (90 °C, μW), **3** was afforded as a single regioisomer. Interestingly, employing *aqueous* DMF a 30% yield of **3** was returned; however, switching to *anhydrous* DMF afforded a significantly improved 60% yield of **3** in 10 min.

Confident that our protocol was robust, we repeated the [3 + 2]-dipolar cycloaddition chemistry using **1** and three readily synthesized α-amino acid derivatives, i.e., *N*-trityl-*O*-propargyl-(*S*)-serine ethyl ester, *N*-Boc-(*S*)-alanine propargyl ester, and *N*-Boc-(*S*)-propargylglycine ethyl ester. We were delighted that the *N*-trityl (**4**) and the thermally labile *N*-Boc group[9] (**5** and **6**) remained intact throughout the reaction affording **4**−**6** (Figure 1) in good (40%) to excellent yields (83% and 89%, respectively).

Scheme 2. Microwave-Mediated "Click" Synthesis of Calix[4]arene **3**



Substituting **1** for *O*-*n*-propoxy-1,3-diazidomethylenecalix-[4]arene and employing the same reaction conditions and catalysts (Scheme 2), a series of 1,3-di-*N*,*C*-protected-α-amino acid hybrid calix[4]arenes (Figure 2) were synthesized using either *N*-Boc, *N*-Fmoc-*O*-propargyl-(*S*)-tyrosine methyl esters or *N*-trityl-*O*-propargyl-(*S*)-serine ethyl ester as the alkyne starting materials. The hybrid calix[4]arenes were

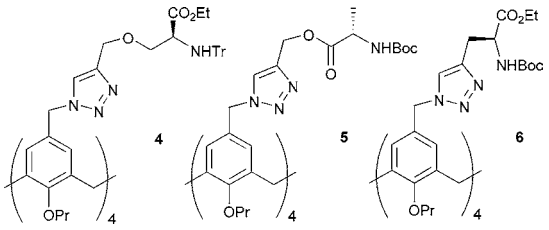


Figure 1. Hybrid calix[4]arenes **4**−**6** synthesized from **1**.

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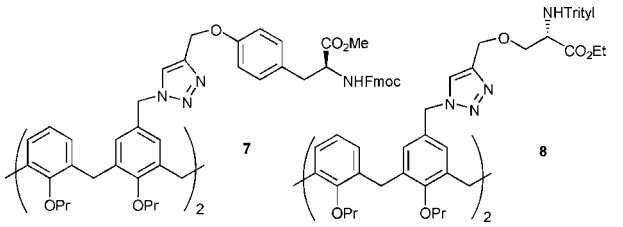


Figure 2. 1,3-Di-(*S*)-α-amino acid hybrid calix[4]arenes **7** and **8**.

afforded in 47% (**7**), 30% (**8**), and 49% (**11**) yields, respectively.

Exemplifying our "click" protocol, the incorporation of bulkier dipeptides onto the upper rim was considered important. Utilizing **1** and *O*-*n*-propoxy-1,3-diazidomethylenecalix[4]arene as starting materials, the respective appendage of four (*N*-Boc-(*S*)-alanine)-*O*-propargyl-(*S*)-tyrosine methyl ester and two (*N*-Boc-(*S*)-valine)-*O*-propargyl-(*S*)-tyrosine methyl ester dipeptides was attempted. The desired tetra- and dipeptide derived hybrid calix[4]arenes **9** and **10** were afforded in 58% and 60% yields, respectively (Figure 3).

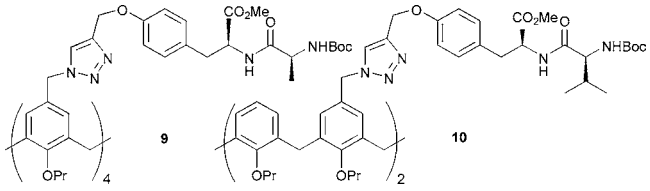
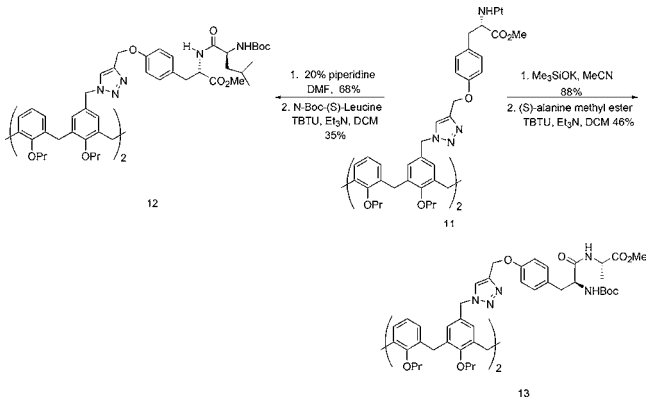


Figure 3. "Click"-appended dipeptide hybrid calix[4]arenes.

Although utilizing *presynthesized* alkyne derived dipeptides as starting materials worked well viz. the synthesis of **9** and **10** (Figure 3), one of our key objectives and an important consideration for future applications was the diversification of the upper-rim appended α-amino acids while attached to the calix[4]arene. The chemoselective cleavage of either a *N*- or *C*-α-amino acid protecting group would considerably enhance our methodology allowing additional and different substrates to be appended thus diversifying and elaborating the calix[4]arenes. The chemoselective cleavage of the *N*-protecting Fmoc group of **7** was readily undertaken (20% piperidine in DMF) affording amine in a 68% yield. The subsequent reaction of this amine with *N*-Boc-(*S*)-leucine afforded **12** in 35% yield. Similarly hydrolysis of the methyl ester on **11** (Pt = Boc) using either potassium trimethylsilanoate in acetonitrile (88% yield) or alternatively lithium hydroxide in aqueous THF (58% yield) afforded (after neutralization) the free acid. Subsequent reaction with (*S*)-alanine methyl ester afforded the corresponding hybrid dipeptide calix[4]arene **13** in 46% yield (Scheme 3).

Advances in glycobiology point toward multivalent carbohydrate−protein and carbohydrate−carbohydrate interactions (glycoside cluster effects) as key drivers of many

Scheme 3. Chemoselective *N*- or *C*-Deprotection of Hybrid Calix[4]arenes



important biological events.[10] Although significant efforts have been undertaken to explain the "cluster effect", the mechanism remains unclear. We deliberated the possibility of appending carbohydrates to the upper-rim of a calix[4]arene using our "click" methodology.[11] Concerned that appending four sugars in close proximity on the upper rim may be problematic (adverse steric effects), initial studies focused on using *O*-*n*-propoxy-1,3-diazidomethylenecalix[4]arene and readily synthesized *O*-propargyl-3,4,5,6-tetra-*O*-acetyl-*β*-D-glucopyranoside or *O*-propargyl-2,3,4,6-tetra-*O*-*β*-D-galactopyranosyl-(1,4)-*O*-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranoside. Employing our standard reaction conditions, we were delighted that the corresponding 1,4-disubstituted-[1,2,3]-triazole disaccharide **14** and 1,3-bis(disaccharide) **15** were afforded in excellent 74% and 88% yields, respectively (Figure 4). That **14** and **15** were in the *β*-configuration was

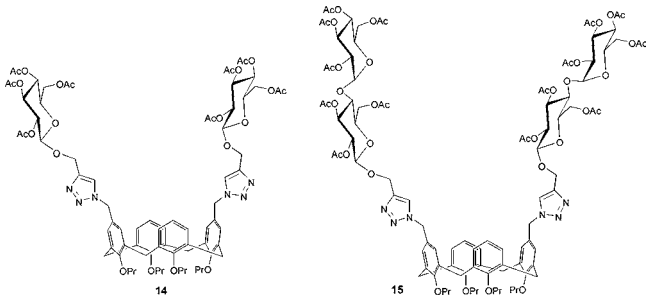


Figure 4. Sugar appended hybrid calix[4]arenes.

confirmed via their ^{1}H NMR spectra and more specifically the $J_{1,2}$ coupling constants (7.3 and 7.9 Hz, respectively). Tornoe et al. have established that copper(I)-catalyzed "click" [3+2]-dipolar cycloadditions afford exclusively the 1,4-disubstituted [1,2,3]triazoles.[12] Gratifyingly, the ^{13}C NMR

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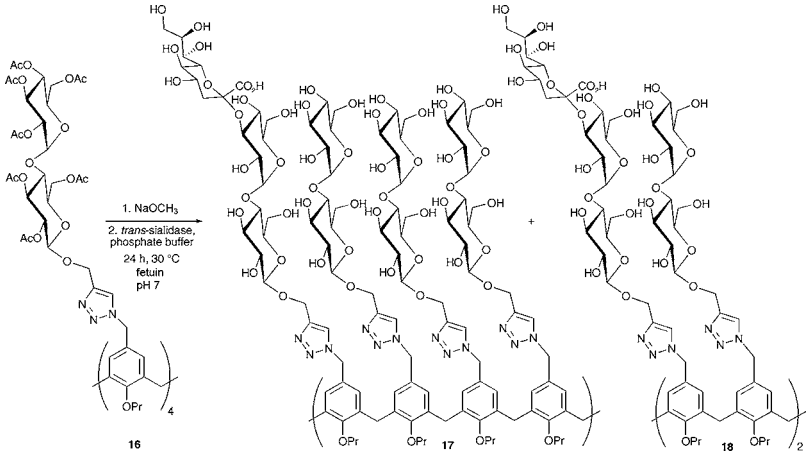
Scheme 4. Chemo-enzymatic Synthesis of Hybrid Calixarenes



data associated with C-5 atom of the [1,2,3]triazole ring of **14** and **15** (δ_C 144.1 and 144.2 ppm, respectively) clearly indicated the formation of a single regioisomer within each reaction.

Multivalent interactions are widespread in biology and, although not fully understood, have fascinating characteristics that distinguish them from their monovalent counterparts.[13] The synthesis of structurally unique entities, capable of probing multivalent interactions is of importance.[14] Within the realm of multivalent interactions sialic acid derived carbohydrates play a pivotal role. Field et al. have demonstrated the chemo-enzymatic synthesis of α-2,3-sialylated octyl lactosides using recombinant *Trypanosoma cruzi trans*-sialidase to effect the key sialylation reaction.[15] Employing a similar procedure, using deacetylated 1,3-bis(lactose) **15**, *trans*-sialidase, and fetuin, initial results were not encouraging. No evidence for either the mono- or 1,3-bis(sialylated lactose)calix[4]arene hybrids was observed. This was attributed to the low solubility of **15** in the aqueous buffer reaction medium. Attempting to negate this the reaction was repeated but with buffer supplemented with 5% DMF. Again no evidence of the desired product(s) was found. Envisaging that octasaccharide **16** (Scheme 4) would have increased solubility we dissolved 2 mg of deacetylated **16** in pH 7 buffer (200 μL) affording a clear solution. The reaction was incubated at 30 °C, quenched and centrifuged. When excess protein and salts were removed, we were delighted to observe the formation of **17** (*m*/*z* 2670.2) and bis-sialylated **18** (*m*/*z* 2983.2) in addition to a small amount of deacetylated **16** (*m*/*z* 2356.1). This important result is the first chemo-enzymatic synthesis of a complex upper-rim hybrid calix[4]arene.

In summary, we have verified that "click" chemistry is ideally suited to the efficient attachment of α-amino acids and carbohydrates onto the upper rim of calix[4]arenes. Of particular value we show that these important adducts can be further manipulated; i.e., the α-amino acid motifs can in situ be efficiently transformed via a series of reactions into calix[4]arenes appended with multiple amino acids. We have also demonstrated, for the first time, the utility of performing chemo-enzymatic procedures for the facile appendage of sialic acids to upper rim appended carbohydrate derived calix[4]arenes. This result paves the way for additional chemo-enzymatic procedures to be utilized on upper-rim appended calixarenes allowing their potential within the biological arena to be fully explored.

Acknowledgment. Support from the University of East Anglia, Dovetrust, Wellcome Trust, and the EPSRC is acknowledged. Professor R. A. Field and Dr. M. Rejzek (JIC, UK) are thanked for help with the chemo-enzyme work and Professor Floris P. J. T. Rutjes, University of Nijmegen, and Chiralix for a gift of (*S*)-propargylglycine.

Supporting Information Available: Experimental procedures and analytical data for **1** and **3**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL071047T

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