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Total Synthesis and Anticancer Activity of (+)-Hypercalin C and Congeners

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Abstract: *The hypercalins are dearomatized acylphloroglucinols with a pendant complex cyclopentane ring that exhibit activity against several cancer cell lines. We report the first total synthesis of (+)-hypercalin C employing a convergent strategy that enabled the dissection of the essential structural features required for the observed anticancer activity. A strategic disconnection involving an unusual C_{sp^3}–C_{sp^2} Suzuki–Miyaura coupling with an α-bromo enolether also revealed an unexpected C−H activation. This strategy targeted designed analogues along the synthetic route to address particular biological questions. These results support the hypothesis that hypercalin C may act as a proton shuttle with the dearomatized acylphloroglucinol moiety being essential for this activity.*

The hypercalins and chinensins are members of the dearomatized, polyprenylated polycyclic acylphloroglucinol (PPAP) family of natural products isolated by Hostettmann from *Hypericum calycinum L* (Figure 1).[1] Several PPAPs

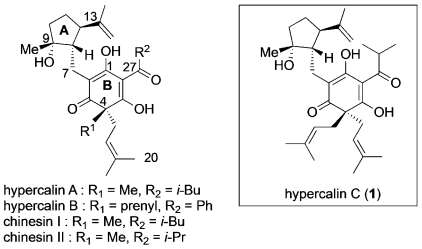

Figure 1. Members of the dearomatized, polyprenylated acylphloroglucinol family, the hypercalins, and chinensins including hypercalin C (**1**).

undergo further oxidative cyclizations leading to polycyclic members that have attracted considerable synthetic interest;[2] however, the hypercalins and chinensins have not yet been synthesized. These natural products display minor structural differences, namely, substitution at the quaternary carbon center (C4, R^1) and the acyl group (C27, R^2). Members of this family possess a range of biological activities including antibacterial, antiviral, and cytotoxicity.[3] For example, hypercalins A–C showed growth-inhibitory activity against the Co-115 human colon carcinoma cell line with ED_{50} values ranging from 0.60–0.83 μM.[1a]

In terms of anticancer activity, hypercalin C is one of the most potent members of this family.[1a] We therefore targeted this congener and designed analogues for synthesis toward the goal of interrogating its proposed mode of action as a cellular membrane proton shuttle.[4]

In this retrosynthetic analysis, disconnections were considered that would allow us to answer biological questions regarding the cytotoxicity of the hypercalins. Thus, a key disconnection involved a C_{sp^3}–C_{sp^2} Suzuki–Miyaura coupling[5] between the boronic monoester **4**,[6] derived from the previously described β-lactone **6**, and bromoenol ether **5** to provide adduct **3** (Figure 2a). This disconnection enables the dissection of the two principal fragments, the cyclopentyl moiety **A** and the dearomatized acylphloroglucinol moiety **B** (Figure 2b), which would allow us to separately assess their cytotoxicity. A subsequent site-selective allylic oxidation could deliver *bis*-methyl hypercalin C, **2**. The requisite cyclopentyl moiety, given its stereochemical complexity, may play an important role in cellular target recognition or simply serve as a hydrophobic group. This could be assessed by coupling with simplified hydrophobic groups (R = alkyl group; Figure 2b) derived from boronic acid **4** accessible from β-lactone **6** in turn derived from carvone.[7] The coupling

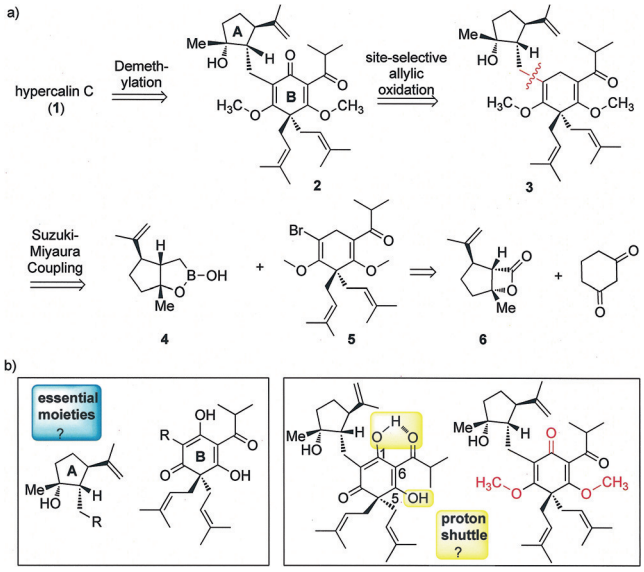

Figure 2. a) Retrosynthetic analysis of hypercalin C and b) targeted derivatives, including the cyclopentyl moiety **A** and the dearomatized acylphloroglucinol moiety **B**, guided by biological questions to be answered in the course of the total synthesis.

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partner for the key coupling event, α-bromo enol ether **5**, could be prepared from 1,3-cyclohexanedione.

A proton shuttle, or protonophore, disrupts the pH gradient across a cell membrane by transporting protons from outside the cell to the higher pH environment inside the cell.[4,8] We planned to test the hypothesis that hypercalin C exerts its bioactivity via this mechanism (Figure 3), which is

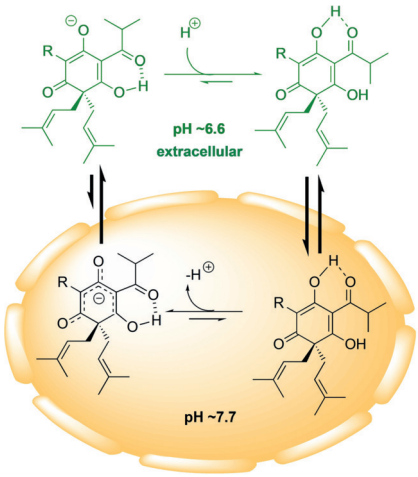
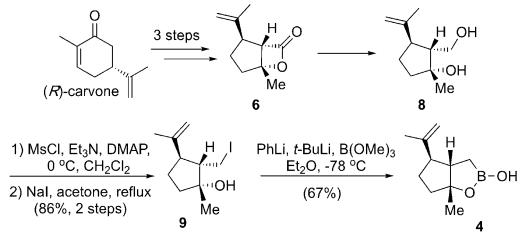


Figure 3. Proton shuttle hypothesis for the cytotoxicity of the hypercalins.

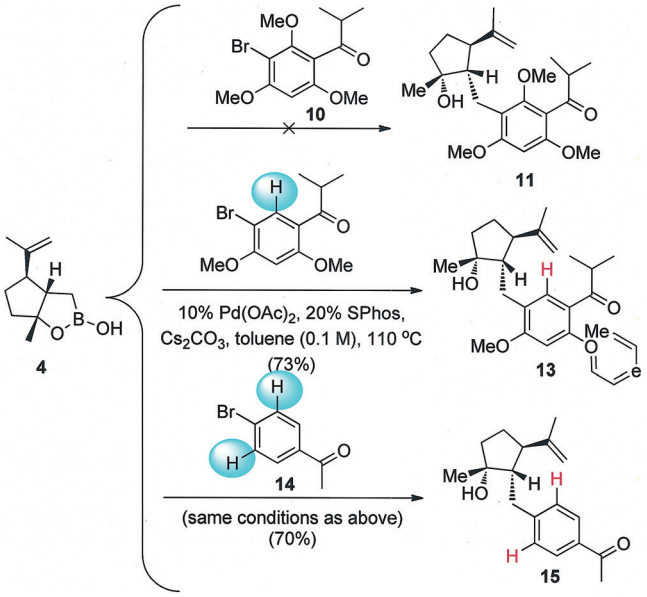
enabled by the highlighted, intramolecularly hydrogen bonded, and relatively acidic C1 and C5 enol hydroxyls (yellow boxes, Figure 2b) and the resulting highly delocalized, anionic species that would be expected to readily traverse the cell membrane (Figure 3). We therefore targeted the *bis*-enol ether **2**, which is also a convenient synthetic precursor to the targeted *bis*-enol, and removes the ability of these molecules to transport protons across the cell membrane, while also minimizing steric perturbations.

We first targeted the synthesis of a versatile C_{sp^3}–C_{sp^2} Suzuki–Miyaura, cyclopentyl coupling partner and chose the bicyclic boronic monoester **4**, building on the prior successful application of this type of coupling partner (Scheme 1).[9] The synthesis commenced with the previously described diol **8**, available in four steps from (*R*)-carvone, through a nucleophile-catalyzed aldol-β-lactonization (NCAL) process, which proceeds with high diastereoselectivity (dr > 19:1) to deliver an intermediate β-lactone **6** setting the two additional stereocenters required for the cyclopentyl moiety of the hypercalins.[10] Selective mesylation of the primary alcohol, followed by a Finkelstein reaction,[11] provided iodide **9** readied for alkylithium generation. Since lithium–halogen exchange is known to be faster than deprotonation,[12] iodide **9** was pretreated with PhLi to deprotonate the tertiary alcohol, and avoid intramolecular quenching. Subsequent addition of *t*-BuLi and quenching with $B(OMe)_3$ delivered the desired cyclopentyl cyclic boronic monoester **4**.



Scheme 1. Synthesis of the cyclopentyl, cyclic boronic monoester **4**.

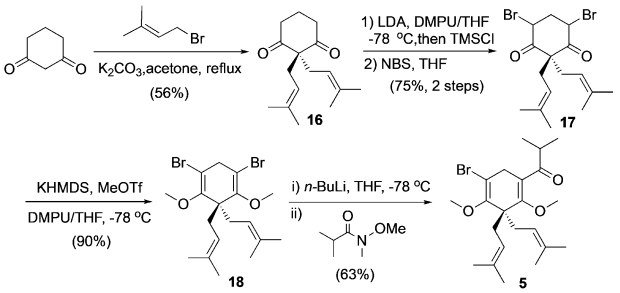
Since transition metal-couplings with *ortho*-disubstituted arenes can be challenging,[13] model studies utilizing boronic monoester **4** and various aryl bromides were undertaken (Scheme 2). Use of less-substituted halogenated arenes would



Scheme 2. Model studies for the cross-coupling with cyclopentyl boronic monoester **4**, leading to simplified, aromatic variants of the cyclohexyl trione core.

also provide simplified ring-A hypercalin C derivatives to inform structure–activity relationships (SAR). Screening of more than 50 different reaction conditions with *tris*-methoxy aryl bromide **10**, failed to provide the desired adduct **11**, a species containing most of the structural features of the natural product. The steric hindrance caused by the *bis-ortho* substitution of the aromatic substrate is known to dramatically impede the reductive elimination step,[14] leading to a competing β-hydride elimination, as evidenced through the isolation of the exocylic alkene, resulting from dehydroboronation of boronic monoester **4,** and the reduced arene derived from bromoarene **10**. We anticipated that the removal of one of the *ortho*-substituents would facilitate the reductive elimination step by minimizing β-hydride elimination and enable subsequent cross-coupling. Indeed, boronic monoester **4** coupled efficiently, under conditions reported by Buchwald and co-workers,[15] with the less-substituted aromatic bromides **12** and **14** with at least one open *ortho* position.

Having identified successful cross-coupling conditions, we targeted the synthesis of an appropriately substituted coupling partner (i.e. vinyl bromide **5**), aware of steric issues



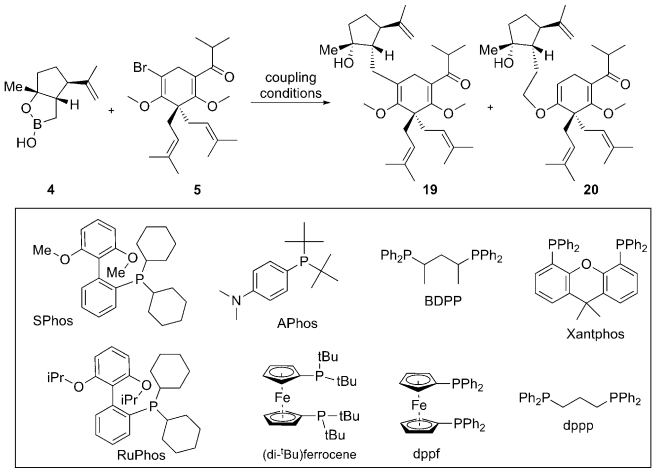
Scheme 3. Synthesis of the Suzuki–Miyaura coupling partner, *bis*-α-bromo enol ether **5**.

identified during model studies (Scheme 3). Alkylative *bis*-prenylation of 1,3-cyclohexanedione provided the dialkylated dione **16**.[16] The subsequent conversion to the highly unstable dibromo diketone **17** was achieved through bromination (freshly recrystallized NBS) of the derived *bis*-silyl enol ether (freshly distilled TMSCl) and the immediate conversion to the *bis*-enolether **18** through a double α-deprotonation/*O*-methylation sequence. This process required extensive optimization since the light-sensitive dibromide **17** was, not surprisingly, highly susceptible to side reactions upon deprotonation, likely stemming from α-elimination, leading to carbene generation. Following extensive experimentation, slow, inverse addition of a THF solution of dibromide **17** to a mixture of high-quality KHMDS (a relatively new/recently opened bottle) and MeOTf in DMPU/THF (1:20) at −78 °C was found to be key for the reproducible production of the *bis*-enolether **18**. Finally, a mono lithium–halogen exchange was achieved by careful control of *n*-BuLi stoichiometry at low temperature to deliver a vinyllithium species that was reacted with the Weinreb amide, derived from 2-methyl propionic acid, to afford the desired keto vinyl bromide **5**.

Our initial studies of the key Suzuki–Miyaura coupling with vinyl bromide **5** and boronic monoester **4,** employing the conditions previously optimized for arene **12,** did not deliver the expected adduct **19,** but rather the C_{sp^3}–C_{sp^3} cross-coupled product **20** (30% yield) via a presumed C–H insertion into the adjacent methoxy group (Table 1, entry 1). The observed C–H activation of an enol ether is, to our knowledge, unprecedented, and this may be due to the fact that α-bromo enol ethers are not frequently used as substrates for cross-coupling reactions. However, the insertion into C–H bonds adjacent to oxygen atoms is well-precedented.[15b]

Building on literature precedent for insertions into C–H bonds adjacent to oxygen atoms,[15b] we propose that a Pd^{II} intermediate **II** is formed following oxidative addition[17] and, due to sterics induced by the adjacent quaternary center and the bulky nature of the SPhos ligand, the methoxy group is forced closer to the Pd-center, facilitating an agostic interaction with the C–H bonds of the methyl group, which, at elevated temperatures, can lead to C–H insertion, generating a Pd^{IV} species **III**. The C_{sp^3} nature of the boronic monoester **4** may also decrease the rate of transmetalation, enabling C–H insertion to be competitive with transmetalation. Reductive elimination would presumably be facilitated by the release of severe steric compression through insertion, leading to the

Table 1: Optimization of the key Suzuki–Miyaura coupling of vinyl bromide **5** and boronic monoester **4**.[a]

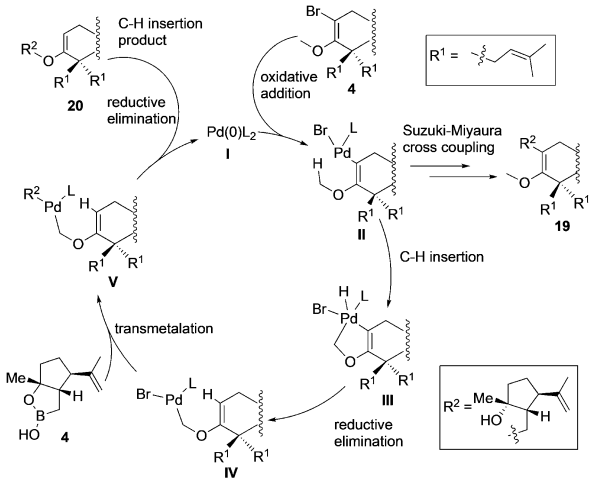


Entry	Ligand (0.2 equiv)	Base (3 equiv)	Suzuki–Miyaura coupling[b]	C–H insertion[b]
1	SPhos[c]	Cs_2CO_3	trace	30%
2	SPhos[d]	Cs_2CO_3	trace	51%
3	SPhos	Cs_2CO_3	21%	30%
4	Xantphos	Cs_2CO_3	trace	21%
5	dppp	Cs_2CO_3	trace	trace
6	dppf	Cs_2CO_3	trace	17%
7	RuPhos	CsF	47%	trace
8	SPhos	K_2CO_3	34%	22%
9	SPhos	K_3PO_4	10%	17%
10	APhos	Cs_2CO_3	40%	12%
11	APhos[e,f]	Cs_2CO_3	74%	trace

[a] Standard conditions: 10% $Pd(OAc)_2$, ligand (0.2 equiv), base (3 equiv), PhMe (0.1 M), 65 °C. [b] Yields refer to purified, isolated products. [c] Reaction temperature was 110 °C. [d] Concentration: 0.01 M. [e] Slow addition of the catalyst (0.1 mL h^{-1}, over 3 h) as a toluene solution. [f] Concentration: 0.2 M.

Pd^{II}-intermediate **IV**. Since β-hydride elimination is not possible at this stage, the longevity and relative stability of intermediate **IV** enables subsequent transmetalation with boronic monoester **4,** which, following reductive elimination, delivers the observed CH-insertion adduct **20** (Scheme 4).

To find optimal conditions for both the desired Suzuki–Miyaura cross-coupling and unexpected C–H insertion, we considered the factors likely impacting these reaction pathways. Most importantly, since C–H activation of the methoxy group is likely driven by the extreme steric environment caused by the quaternary center in both the substrate and the ligand, we anticipated that replacement of SPhos with a smaller ligand would drive the reaction to the desired C_{sp^3} Suzuki–Miyaura coupling. On the other hand, to promote the oxidative addition of palladium to the rather electron-rich bromo enol ether, the ligand employed should be electron rich and bulky, which in turn also promotes the final reductive elimination. At the same time, a considerable energy barrier is present for C–H insertion, thus the high temperature favors this side reaction. Indeed, when the temperature was lowered from 110 to 65 °C, the desired Suzuki–Miyaura coupled product **19** was obtained in 20–30% yield, while the C–H insertion product, ether **20**, was still obtained in 30% yield

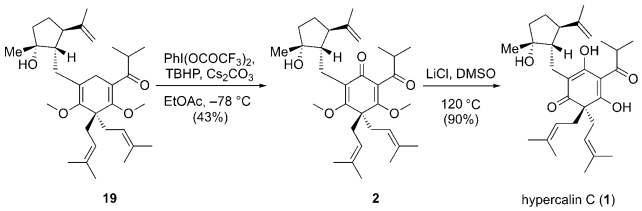


Scheme 4. Proposed catalytic cycle leading to typical Suzuki–Miyaura coupling and an unexpected C–H insertion into an enol ether.

(Table 1, entry 3). Furthermore, the intramolecular nature of the C–H insertion process makes reaction concentration a key factor and it was surmised that lower concentration would favor the C–H insertion pathway. With the above considerations and extensive screening, including the variation of ligands and base (Table 1, entries 3–11; for other conditions examined, see Table S1 in the Supporting Information), we ultimately found reaction conditions that favored the desired Suzuki–Miyaura coupling using APhos as ligand (74%, Table 1, entry 11), while alternative conditions employing SPhos at low concentration gave primarily the C–H insertion product **20** (51% yield, Table 1, entry 2).

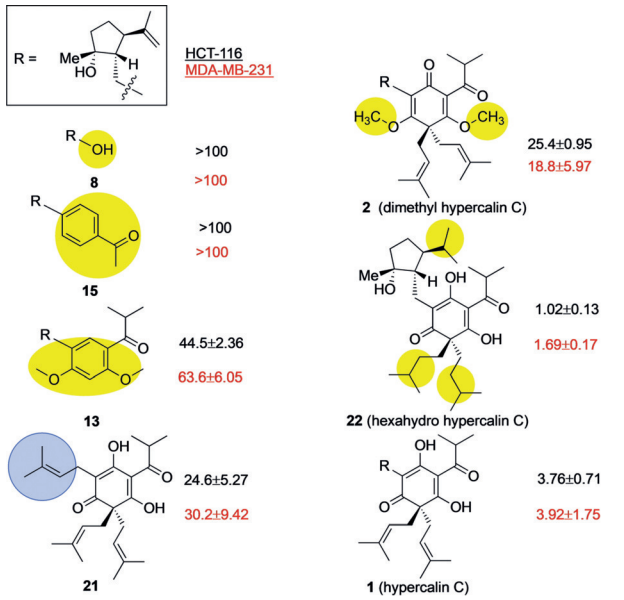
The final steps to the targeted dimethyl hypercalin C and the natural product, hypercalin C, required a selective allylic oxidation, followed by demethylation for the latter product. This oxidation required site-selectivity, given the presence of two prenyl groups and an isobutenyl moiety presenting nine potential oxidizable allylic positions with most positions being less hindered than the targeted allylic position. However, electronic effects of the targeted *bis*-allylic position were expected to favor a site-selective oxidation. This led us to oxidative methods that involved initial hydrogen atom abstraction since the resulting *bis*-allylic radical would be stabilized.[18] We were gratified to find that conditions reported by Shair in their synthesis of hyperforin,[19] involving the generation of a peroxide radical for hydrogen atom abstraction at the most reactive allylic site (weakest C–H bond) and subsequent oxidation, provided a 43% yield of the desired ketone **2** (Scheme 5). Demethylation of dimethyl hypercalin C (**2**) was achieved with LiCl in DMSO at elevated temperature providing clean conversion to hypercalin C (**1**) in 90% yield.

We measured the cytotoxicity of synthetic hypercalin C and its derivatives against HCT-116 colon cancer and MDA-MB-231 breast cancer cell lines (Table 2). The bare cyclopentyl moiety bearing an additional alcohol **8** or aromatic moieties in place of the cyclohexanetrione, namely arene derivatives **15** and **13**, were not cytotoxic up to 100 μM. These



Scheme 5. Completion of the total synthesis of hypercalin C (**1**).

Table 2: Cytotoxicity of hypercalin C and derivatives against HCT-116 (in black) and MDA-MB-231 (in red) cell lines (IC_{50}, μM).



IC_{50} values were determined for the indicated compounds with MDA-MB-231 and HCT-116 cells in vitro. Standard error of the mean represents the variation of three biological replicates, each with at least three technical replicates.

results suggest that the cyclopentyl moiety alone, though rich in stereochemical information, does not significantly contribute to the observed cytotoxicity. This is further supported by derivative **21**,[20] which retains some of the original cytotoxicity (6.5–7.7X decrease) while only possessing a simple isopentyl chain in place of the complex cyclopentane found in hypercalin C. Interestingly, the presence of oxygen in the arene ring, see derivative **13**, but a lack of acidic protons led to the initially observed cytotoxicity (at less than 100 μM). The cytotoxicity of synthetic hypercalin C was found to be in the 3–4 μM range, consistent with previous reports;[1a] however, dimethyl hypercalin C (**2**) showed a measurable decrease in cytotoxicity (approximately 5–7X). While this lends some credence to the proposed requirement of acidic enols for cytotoxicity, the difference is not significant enough to exclude alternative hypotheses regarding the mode of action of the hypercalins. On the other hand, the hydrogenated derivative **22** showed similar activity to the natural product, indicating that the unsaturation of the prenyl side chains has minimal effect on cytotoxicity.

In conclusion, the first total synthesis of hypercalin C was achieved in 10 steps (longest linear sequence from carvone),

with an overall yield of 8%. The synthetic sequence featured a key C_{sp^3}–C_{sp^2} Suzuki–Miyaura coupling with an uncommon α-bromo enol ether coupling partner and a boronic monoester derived from carvone. This disconnection represents a formal α-alkylation of a ketone via transition metal coupling, offering a unique perspective for ketone α-functionalization. Furthermore, we discovered an unexpected, and conceptually novel, C–H insertion/Suzuki–Miyaura coupling process employing the α-bromo enol ether substrate. Guided by the desire to answer particular biological questions regarding SAR enabled by the designed synthetic strategy, several hypercalin C analogues were accessed and tested against two cancer cell lines for cytotoxicity. Some support for the proton shuttle hypothesis for hypercalin C was garnered through these studies, however the modest decrease in cytotoxicity observed when the acidic enol protons were substituted for methyl groups suggests that other cellular mechanisms for the observed cytotoxicity of the hypercalins are likely operative.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: C–H insertion · chiral pool · protonophore · Suzuki–Miyaura coupling · β-lactone

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