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Total Synthesis and Bioactivity of Resolvin E2

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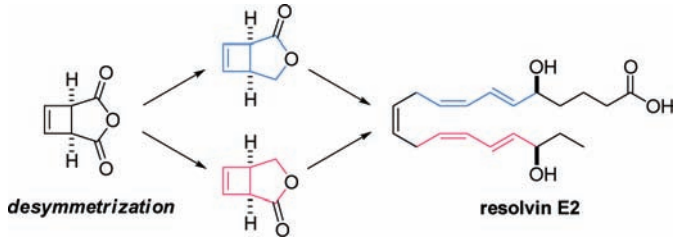
ABSTRACT



Resolvin E2 is a potent anti-inflammatory compound, derived from eicosapentaenoic acid. The efficient total synthesis of resolvin E2 by taking advantage of its intrinsic pseudoenantiomeric substructures is reported. The synthetic resolvin E2 proved to be biologically active in blocking neutrophil infiltration and reducing proinflammatory cytokines in the acute peritonitis model.

Resolvins are a new family of lipid mediators derived from omega-3 polyunsaturated fatty acids, namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are generated during the resolution phase of acute inflammation.[1] Resolvin E1 is biosynthesized from EPA via cyclooxygenase (COX)-2- and 5-lipoxygenase-mediated conversion and has been shown to possess significant anti-inflammatory and proresolution properties, thereby protecting organs from collateral damage.[2] Another E series resolvin, namely, resolvin E2 (**1**), is formed via reduction of 5*S*-hydroperoxy-18*R*-hydroxy-EPE, an intermediate in the biosynthesis of resolvin E1, and exhibits potent anti-inflammatory properties in murine peritonitis.[3] It has been hypothesized that these E series resolvins contribute to the beneficial actions that have been attributed to EPA in certain human diseases, particularly those in which inflammation is suspected as a key component in pathogenesis. Motivated by their therapeutic potential for new treatment of human disorders associated with aberrant inflammation, we launched the synthetic studies of resolvins as well as other lipid mediators. Here we report an efficient total synthesis of resolvin E2[4] and its biological activity in reducing neutrophil infiltration and proinflammatory cytokine productions in vivo.

We planned to simplify the synthetic route to resolvin E2 (**1**) by taking advantage of its two symmetric substructures at C5−10 and C13−18 (Scheme 1). Retrosynthetic disconnections at C10−11 and C12−13 provided a C11−12 unit together with pseudo-enantiomeric fragments, **2** and **3**, both of which have the *E*,*Z*-conjugated olefin and allylic alcohol groups. Because of their structural similarity, **2** and **3** would be prepared from enantiomers of **6** by applying the same

(1) For reviews, see: (a) Serhan, C. N.; Chiang, N.; Van Dyke, T. E. *Nat. Rev. Immunol.* **2008**, *8*, 349–361. (b) Serhan, C. N.; Chiang, N. *Br. J. Pharmacol.* **2008**, *153*, S200–S215.

(2) (a) Arita, M.; Bianchini, F.; Aliberti, J.; Sher, A.; Chiang, N.; Hong, S.; Yang, R.; Petasis, N. A.; Serhan, C. N. *J. Exp. Med.* **2005**, *201*, 713–722. (b) Schwab, J. M.; Chiang, N.; Arita, M.; Serhan, C. N. *Nature* **2007**, *447*, 869–875.

(3) Tjonahan, E.; Oh, S. F.; Siegelman, J.; Elangovan, S.; Percarpio, K. B.; Hong, S.; Arita, M.; Serhan, C. N. *Chem. Biol.* **2006**, *13*, 1193–1202.

(4) Total syntheses of resolvins E1 were disclosed in ref 2a. Total synthesis of resolvins D2 and D5 were reported. (a) Rodriguez, A. R.; Spur, B. W. *Tetrahedron Lett.* **2004**, *45*, 8717–8720. (b) Rodriguez, A. R.; Spur, B. W. *Tetrahedron Lett.* **2005**, *46*, 3623–3627. For a review of syntheses of eicosanoids, see: (c) Nicolaou, K. C.; Ramphal, J. Y.; Petasis, N. A.; Serhan, C. N. *Angew. Chem., Int. Ed.* **1991**, *30*, 1100–1116.

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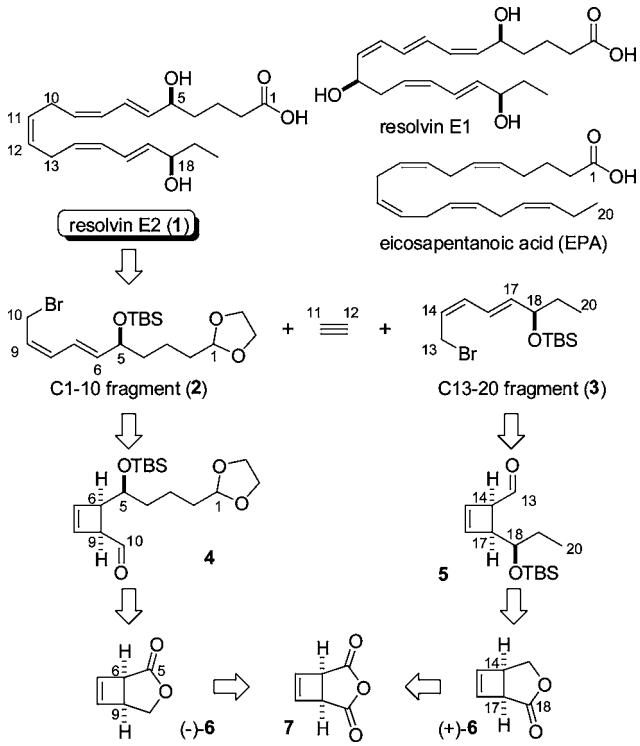
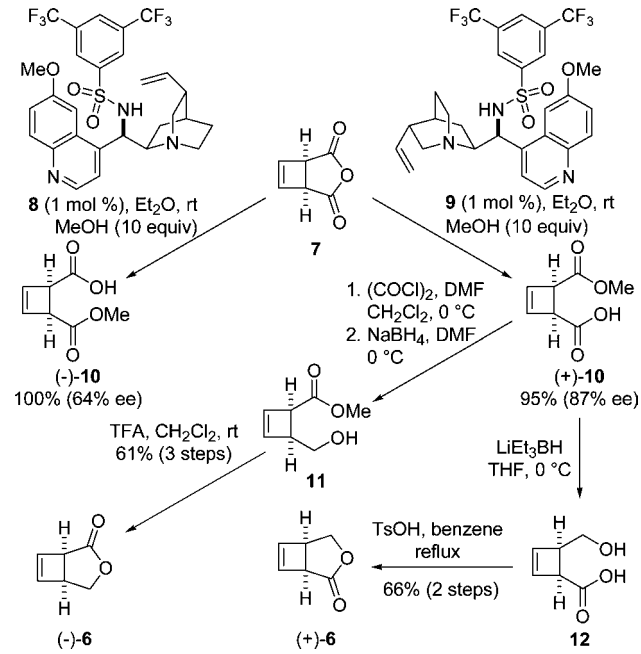
Scheme 1. Structures of Resolvins and EPA and Retrosynthesis of Resolvin E2



Scheme 2. Synthesis of Both Enantiomers of **6**



strategy. Specifically, the stereocenters at C5 of **4** and C18 of **5** would be generated by substrate-controlled stereoselective addition of the corresponding carbon nucleophiles, while the *E*,*Z*-olefins at C6 of **2** and C17 of **3** would be constructed using a torquoselective thermal electrocyclic ring-opening reaction[5] of cyclobutene aldehydes **4** and **5**, respectively.[6] Hence, the stereochemistries of the cyclobutane of (−)- or (+)-**6** were envisioned to be transferred to the stereochemistries of the hydroxy group at C5 or C18 and the diene at C6 or C17. A pair of optically active six-carbon units **6**[7] would be obtained from the known achiral *meso*-anhydride **7**[8] by enantioselective desymmetrization.

Both enantiomers of **6** were prepared from methyl ester (+)-**10** (Scheme 2).[9] The critical desymmetrization of *meso*-**7** into (+)-**10** was realized using a catalytic amount of the quinine derivative **9**, according to the conditions developed by Song.[10,11] Namely, 1 mol % of **9** and 10 equiv of methanol were applied to **7** in Et_2O to generate (+)-**10** in highly enantioselective fashion (95% yield, 87% ee).[12,13] Interestingly, methanolysis of the same **7** using the quinidine derivative **8**, the pseudo-enantiomer of **9**, indeed gave the enantiomeric (−)-**10**, albeit in lower enantioselectivity (64% ee). Due to this, we decided to synthesize (−)- and (+)-**6** from the same (+)-**10** using chemoselective reduction of either the carboxylic acid or the ester. Lactone (−)-**6** was prepared in three steps: conversion of the carboxylic acid of (+)-**10** into the acid chloride, followed by chemoselective $NaBH_4$ reduction,[14] and subsequent acid-mediated cyclization of methyl ester **11**. The enantiomer (+)-**6** was in turn synthesized by $LiEt_3BH$ reduction[15] of the methyl ester of (+)-**10** and subsequent cyclization of carboxylic acid **12** under acidic conditions.

Synthesis of the C1−10 fragment **2** started with reduction of (−)-**6** by DIBAL-H, followed by addition of Grignard

(5) For rationalization of torquoselectivity, see: (a) Rudolf, K.; Spellmeyer, D. C.; Houk, K. N. *J. Org. Chem.* **1987**, *52*, 3708–3710. (b) Niwayama, S.; Kallel, E. A.; Spellmeyer, D. C.; Sheu, C.; Houk, K. N. *J. Org. Chem.* **1996**, *61*, 2813–2825. (c) Dolvier, W. R., Jr.; Koroniak, H.; Houk, K. N.; Sheu, C. *Acc. Chem. Res.* **1996**, *29*, 471–477.

(6) Wallace demonstrated selective ring opening of cyclobutene aldehydes and substrate-controlled stereoselective introductions of various carbon nucleophiles to disubstituted cyclobutenes: (a) Binns, F.; Hayes, R.; Ingham, S.; Saengchantara, S. T.; Turner, R. W.; Wallace, T. W. *Tetrahedron* **1992**, *48*, 515–530. (b) Hodgetts, K. J.; Wallis, C. J.; Wallace, T. W. *Tetrahedron Lett.* **1994**, *35*, 4645–4648. (c) Hodgetts, K. J.; Wallis, C. J.; Wallace, T. W. *Synlett* **1995**, 1235. (d) Binns, F.; Hayes, R.; Hodgetts, K. J.; Saengchantara, S. T.; Wallace, T. W.; Wallis, C. J. *Tetrahedron* **1996**, *52*, 3631–3658.

(7) Enantioselective syntheses of (+)- and (−)-**6** by enzymatic resolution were reported: Gourdel-Martin, M.-E.; Comoy, C.; Huet, F. *Tetrahedron: Asymmetry* **1999**, *10*, 403–404. See also ref 6b and 6c.

(8) (a) Koltzenburg, G.; Fuss, P. G.; Leitich, J. *Tetrahedron Lett.* **1966**, *29*, 3409–3414. (b) Brauman, J. I.; Archie, W. C., Jr. *J. Am. Chem. Soc.* **1972**, *94*, 4262–4265. (c) Gauvry, N.; Comoy, C.; Lescop, C.; Huet, F. *Synthesis* **1999**, 574–576.

(9) For a review of synthetic applications of lactone **6** and derivatives, see: Gauvry, N.; Lescop, C.; Huet, F. *Eur. J. Org. Chem.* **2006**, *520*, 7–5218.

(10) Oh, S. H.; Rho, H. S.; Lee, J. W.; Lee, J. E.; Youk, S. H.; Chin, J.; Song, C. E. *Angew. Chem., Int. Ed.* **2008**, *47*, 7872–7875.

(11) For a review of asymmetric alcoholysis of cyclic anhydrides, see: Chen, Y.; McDaid, P.; Deng, L. *Chem. Rev.* **2003**, *103*, 2965–2983.

(12) Enantiomeric excess was determined from ^{1}H NMR spectra of the MTPA ester of alcohol **11**.

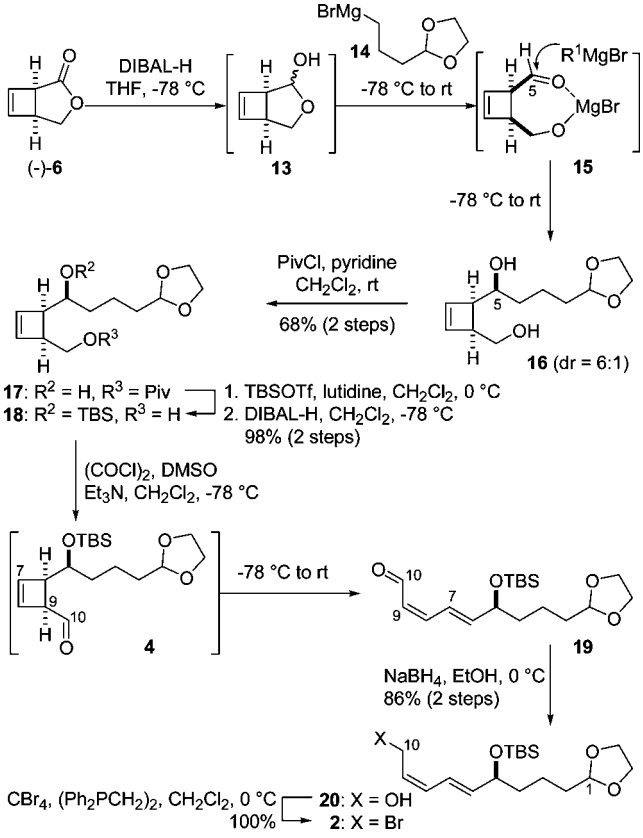
(13) Absolute configuration of lactone **6** was deduced from the report of Wallace and co-workers, see ref 6d.

(14) Fujisawa, T.; Mori, T.; Sato, T. *Chem. Lett.* **1983**, 835–838.

(15) Brown, H. C.; Kim, S. C.; Krishnamurthy, S. *J. Org. Chem.* **1980**, *45*, 1–12.

reagent **14** in one pot,[16] resulting in stereoselective introduction of the C5-hydroxy group of **16** (dr = 6:1, Scheme 3).[17]

Scheme 3. Synthesis of the C1−10 Fragment

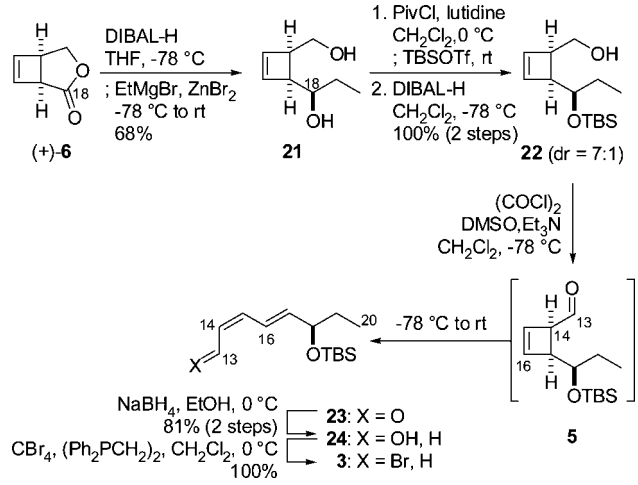


The high diastereoselectivity is attributable to chelation between the magnesium alkoxide and the aldehyde and subsequent nucleophilic attack from the convex face of the 4/7-fused ring system **15**.[6b,d] Next, 1,4-diol **16** was transformed to alcohol **18** by a protection/deprotection procedure: stepwise introduction of Piv and TBS to the primary and the secondary alcohols, respectively, and subsequent reductive removal of the Piv ester from **17**.

Swern oxidation of alcohol **18** at −78 °C generated aldehyde **4**, which underwent the crucial torquoselective electrocyclic ring-opening reaction even at room temperature to deliver *E*,*Z*-diene **19** as a sole isomer.[5] Stereoselective formation of the diene of **19** would originate from strong preference of the electron-accepting aldehyde of **4** for the inward rotation.[6] Because of its chemical instability, the resulting α,*β*,*γ*,*δ*-unsaturated aldehyde **19** was immediately subjected to $NaBH_4$ reduction without purification to give allylic alcohol **20**.[18] Finally, bromination of the chemically sensitive allylic alcohol was realized by the action of CBr_4 and $(CH_2PPh_2)_2$ to give the C1−10 fragment **2**.[19,20]

The C13−20 fragment **3** was synthesized from (+)-**6** similarly to the C1−10 fragment **2** (Scheme 4). Reduction

Scheme 4. Synthesis of the C13−20 Fragment



of (+)-lactone **6** with DIBAL-H was followed by stereoselective addition of ethyl magnesium bromide in the presence of zinc bromide to afford **21** with the desired C18-stereochemistry (dr = 7:1).[21] After protecting group manipulations from **21** to **22** in two steps, Swern oxidation of the primary alcohol of **22** to aldehyde **5** accelerated the thermal ring-opening reaction to produce *E*,*Z*-diene **23** as a single isomer. Reduction of the resulting aldehyde of **23** into alcohol **24**, followed by bromination, led to the C13−20 fragment **3**.

Final convergent assemblies of the three partial structures utilized two copper-mediated couplings (Scheme 5). The bromide of **3** was first displaced with the copper acetylide, generated from ethynyl magnesium bromide and CuCl,[22] delivering the C11−20 fragment **25**. Deprotonation of the C11-proton of **25** by *n*-BuLi in the presence of $CuBr{\cdot}SMe_2$[23] at −78 °C then afforded the corresponding copper acetylide, which was treated with the C1−10 fragment **2**, giving rise to the entire structure **26**. Intriguingly, only this particular condition produced a sufficient amount of the adduct **26**. For instance, use of copper iodide instead of copper bromide for the coupling only gave a mixture of byproducts, in which the C6-*E*,*Z*-olefins were reacted or isomerized.

(16) Wenkert, D.; Ferguson, S. B.; Porter, B.; Qvarnstrom, A.; McPhail, A. T. *J. Org. Chem.* **1985**, *50*, 4114–4119.

(17) Stereochemistries at C5 and C18 positions were unambiguously determined by the modified Moshers' method. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096. See Supporting Information for the detailed procedure.

(18) *E*,*Z*-Dienes **20** and **24** were easily isomerized into *E*,*E*-diene upon standing at room temperature. Thus, organic solution of the reaction mixture from the oxidation was directly used for the reduction.

(19) (a) Leblanc, Y.; Futzsimmons, B. J.; Adams, J.; Perez, F.; Rokach, J. *J. Org. Chem.* **1986**, *51*, 789–793. (b) Schmidt, S. P.; Brooks, D. W. *Tetrahedron Lett.* **1987**, *28*, 767–768.

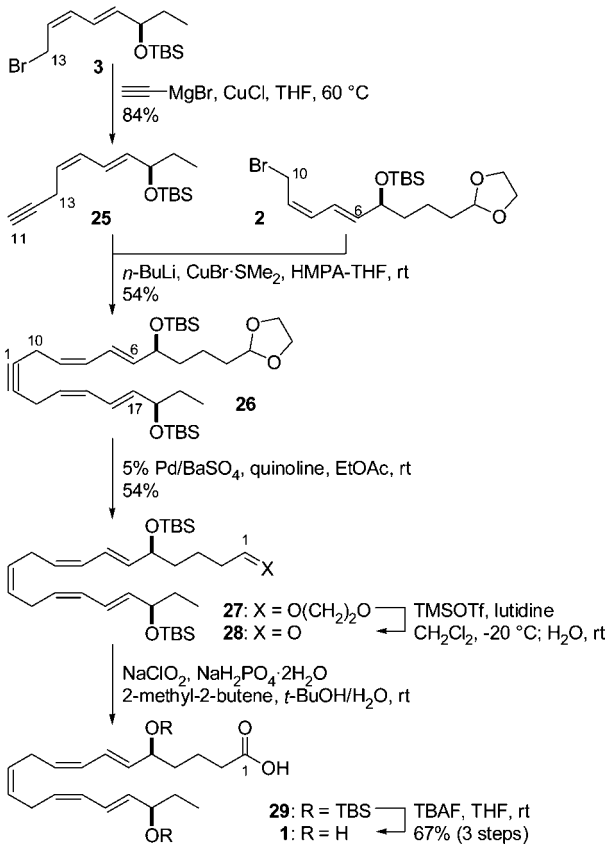
(20) Bromination of alcohol **20** with CBr_4 and Ph_3P gave only trace amount of the product **2**.

(21) Addition of EtMgBr in the presence of $Ti(Oi\text{-}Pr)_3Et$ gave the desired product as a single isomer; however, the yield was 23%.

(22) Bloch, R.; Gasparini, G.; Girard, C. *Chem. Lett.* **1988**, 1927–1930.

(23) (a) Kurtz, P. *Liebigs Ann. Chem.* **1962**, *658*, 6–20. (b) Normant, J. F. *Synthesis* **1972**, 63.

Scheme 5. Total Synthesis of Resolvin E2



Four transformations from **26** led to the targeted resolvin E2 (Scheme 5). Lindlar conditions[24] enabled partial reduction of the alkyne of **26** into alkene **27** without reduction and/or isomerization of the reactive C6- and C17-*E*,*Z*-olefins. Acid-mediated removal of the ketal of **27** was troublesome because of the presence of the acid-labile allylic TBS ethers. After many attempts, we found that Kita's conditions[25] were effective for selective reaction of the cyclic ketal. Treatment of **27** with an excess amount of TMSOTf and lutidine provided aldehyde **28** in high yield. Lastly, $NaClO_2$-mediated oxidation of the obtained aldehyde **28** into a carboxylic acid and subsequent desilylation with TBAF gave rise to resolvin E2 (**1**).

We evaluated the bioactivity of synthetic **1** using the in vivo inflammation model (Figure 1).[3] Zymosan A, a glucan from the yeast cell wall, was used to induce sterile inflammation characterized by local neutrophil infiltration and

(24) Lindlar, H. *Helv. Chim. Acta* **1952**, *57*, 446–450.
(25) Fujioka, H.; Okitsu, T.; Sawama, Y.; Murata, N.; Li, R.; Kita, Y. *J. Am. Chem. Soc.* **2006**, *128*, 5930–5938.

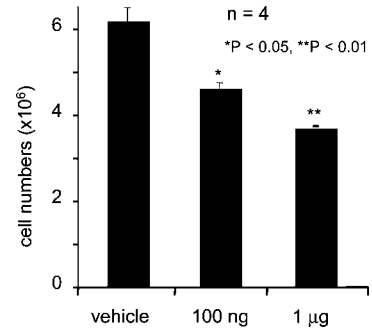


Figure 1. Synthetic resolvin E2 reduced neutrophil infiltrations and cytokine productions in zymosan-induced peritonitis.

proinflammatory cytokine productions. In acute peritonitis with zymosan A, intravenous administration of synthetic resolvin E2 as low as 0.1 or 1.0 μg significantly blocked neutrophil infiltrations at 2 h in the inflamed peritoneal cavity (Figure 1). The potency of resolvin E2's anti-inflammatory action was comparable to that of a higher dose of dexamethasone at 10 μg (data not shown). Also **1** (1.0 μg *i.v.*/mouse) markedly reduced production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α (123.2 ± 15.8 pg vs 56.7 ± 7.3 pg, $p < 0.005$) and interleukin (IL)-6 (3.40 ± 0.29 ng vs 2.05 ± 0.22 ng, $p < 0.02$) (see Supporting Information).

In summary, the efficient total synthesis of resolvin E2 (**1**) was achieved by utilizing the intrinsic pseudoenantiomeric nature of the key fragments **2** and **3**. Most importantly, the two stereochemistries of **6** introduced via the desymmetrization step were effectively transferred to those of the hydroxy group and the diene for preparing **2** and **3**. The obtained fragments were assembled into **1** in a convergent fashion. Further synthetic studies and functional analyses of the resolvins and other lipid mediators are currently underway in our laboratory.

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Supporting Information Available: Experimental procedures and characterization data of newly synthesized compounds and biological assay data of synthetic **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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