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Solid-Phase Synthesis of Trisubstituted Benzo[1,4]-Diazepin-5-one Derivatives

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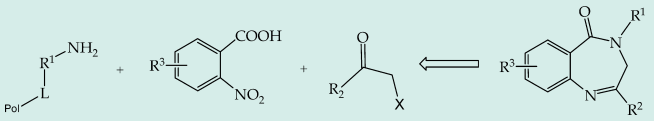
Supporting Information

ABSTRACT: Solid-phase synthesis of 3,4-dihydro-benzo[e]-[1,4]diazepin-5-ones with three diversity positions is described. Various primary amines were used as the starting material and immobilized on the polystyrene resin equipped with different acid-labile linkers. Polymer-supported amines were converted to α-aminoketones with the use of their sulfonylation with the 4-nitrobenzensulfonylchoride (4-Nos-Cl) and subsequent alkylation with α-bromoketones. After the cleavage of the 4-Nos group, the corresponding α-aminoketones were acylated with various *o*-nitrobenzoic acids. Reduction of the nitro group followed by spontaneous on-resin ring closure gave the target immobilized benzodiazepines. After acid-mediated cleavage the products were obtained in very good crude purity and satisfactory yields, which makes the developed method applicable for simple library synthesis of the corresponding derivatives in a combinatorial fashion.



KEYWORDS: *benzodiazepines, solid-phase synthesis, α-aminoketones, nitrobenzoic acids, haloketones*

INTRODUCTION

In the entire history of 1,4-benzodiazepine scaffold containing substances, 5-substituted-1,3-dihydro-benzo[e][1,4]diazepin-2-ones **I** have been studied most extensively particularly because of their influence on a central nervous system (CNS).[1,2] The most frequently observed effects which resulted in an introduction of more than 30 marketed benzodiazepine drugs[3] are sedative-hypnotic,[4] anxiolytic,[5] muscle relaxant[6] and anticonvulsant[7] activities. The commercial success of benzodiazepine drugs (such as clonazepam, diazepam, bromazepam, or flunitrazepam, see Figure 1) caused exhaustive research in this area and preparation of large number of benzodiazepine derivatives have been described. In contrast, structurally isomeric 2-phenyl-3,4-dihydro-benzo[e][1,4]diazepin-5-ones **II** have been studied rarely and only a few articles dedicated to the preparation and properties of such compounds have been published.

Until now three general methods have been developed for the preparation of 2-phenyl-3,4-dihydro-benzo[e][1,4]diazepin-5-ones **II** and all of them take advantage of traditional solution-phase synthesis. The oldest approach is based on the cyclization of phenacyl anthranilamides which can be easily obtained by the reaction of isatoic anhydride and α-aminoacetophenones.[8−11] The second method is based on the ring closure of β-ketoesters with *o*-phenylendiamine and it has been used for the preparation of derivatives which have been studied for their platelet-activating factor (PAF) antagonist activity.[12] The latest synthetic approach takes advantage of the multicomponent Ugi reaction with use of isocyanide chemistry.[13] The last mentioned method has been recently used with slight modification for the preparation of benzodiazepine β-turn mimetics.[14]

In our current research, we have been focused on an extension of the method that uses α-aminoacetophenones as key synthons. Preparation of such compounds in solution is well-known, the traditional approach is based on the reaction of bromoacetophenones with sodium azide[15,16] or urotropine.[17] Quite recently an efficient method involving solid-support chemistry dealing with the synthesis of α-aminoketones and their use for the diversity-oriented synthesis of various nitrogenous heterocycles has been developed.[18,19]

This article describes another application of polymer-supported α-aminoketones for the preparation of 2-phenyl-3,4-dihydro-benzo[e][1,4]diazepin-5-ones from commercially available building blocks. The solid-phase synthesis concept has been used to introduce the methodology applicable for the future preparation of chemical library and subsequent structure−activity relationship studies of the target substances **II** that contain three diversity positions: (i) substitution at position 2, (ii) substitution of the nitrogen atom at position 4, (iii) substitution of a benzene ring of the benzodiazepine scaffold.

RESULTS AND DISCUSSION

The key building blocks for the preparation of the target substances were primary amines, α-bromoketones and *o*-nitrobenzoic acids.

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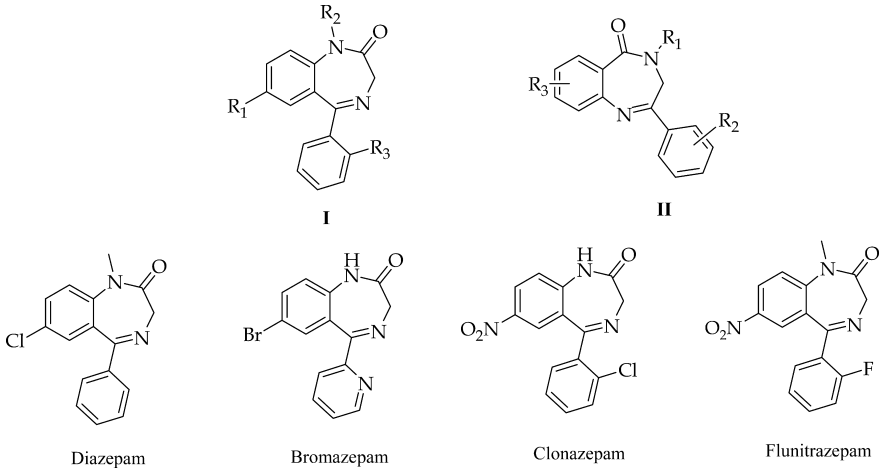
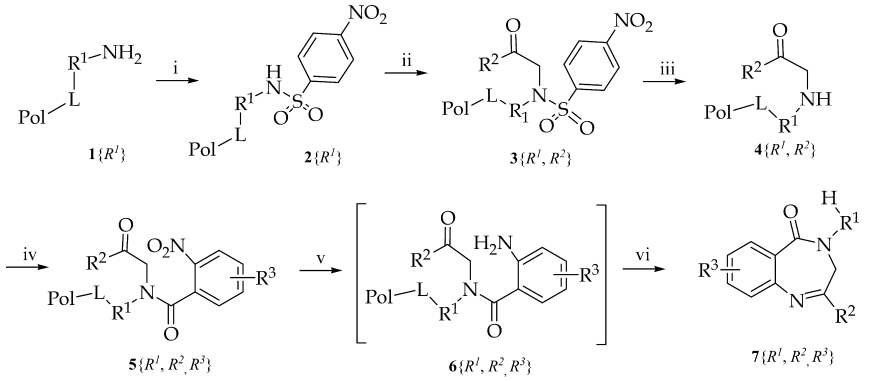


Figure 1. General structure of target substances (II) and benzodiazepine drugs (I).

Scheme 1. General Synthetic Route Leading to the Target Benzodiazepines[a]



[a]Reagents: (i) 4-nitrobenzensulfonyl chloride, 2,6-lutidine, DCM, rt, 16 h; (ii) bromoketone, DIEA DMF, rt, 16 h; (iii) 2-mercaptoethanol, DBU, rt, 10 min; (iv) *o*-nitrobenzoic acids, DIC, DMF, rt, 16 h; (v) $SnCl_2 \cdot 2H_2O$, DIEA, deoxygenated DMF, rt, 16 h (repeated); (vi) 50% TFA in DCM, rt, 30 min.

A general synthetic route leading to target compounds is described in Scheme 1.

To expand the diversity of R^1 position as much as possible, starting amines of various structures were attached to the polystyrene resin *via* suitable acid-labile linkers (Scheme 2). To introduce an aliphatic chain with a terminal hydroxy group, amino group or carboxy group respectively, Wang resin was used and Fmoc-aminoethanol **1**{*1*}, propylendiamine **1**{*2*} and Fmoc-*β*-Ala **1**{3} were immobilized. To introduce an aliphatic ligand with the terminal unsubstituted carboxamide group, Rink amide resin was used and acylated with Fmoc-*β*-Ala-OH to give aminoderivative **1**{*4*}. To include *N*-substituted carboxamide the aminomethylated polystyrene resin equipped with backbone amide linker (BAL) was reductively aminated with two model amines (propylamine and benzylamine) which were subsequently acylated with Fmoc-*β*-Ala-OH to give intermediates **1**{*5*} and **1**{*6*}.

Following the previously described procedure[19] the amines **1**{R^1} were transformed to the corresponding *α*-aminoketones **4**{R^1,R^2}. Surprisingly, sulfonylation of aminoderivatives with 4-nitrobenzensulfonyl chloride was not quantitative in most cases (resins **1**{*1*} and **1**{*3*−*5*}) and the reaction had to be repeated for completion. For verification of the subsequent alkylation we used compound **2**{3} and five aromatic bromoketones substituted with electron-withdrawing as well as electron-donating groups were tested. Also one heterocyclic and one aliphatic haloketone was included (see Figure 2).

Alkylation with bromoketones *1*−*6* afforded sulfonamides **3**{*3*, *1*−*6*} with excellent purity (more than 90%, UPLC-UV traces). A different result was obtained when chloroacetone 7 was used. Alkylation with this agent gave the desired intermediate in limited purity (up to 75%). First, we tried to optimize the reaction conditions with the use of different solvents, bases and temperature (see Table 2) but we did not manage to increase the overall purity. Additionaly, repetition of reaction conditions was tested but the purity decreased due to secondary products formation.

Denosylation of intermediates **3**{R^1,R^2} afforded the corresponding aminoketones **4**{R^1,R^2} that were acylated with *o*-nitrobenzoic acid. It should be noted that denosylation of intermediate **3**{*6*,*1*} required a significantly longer reaction time (60 min instead of typical 10 min procedure). After the acylation step, we observed formation of a side product, which was identified with the use of UPLC-UV-MS as the dealkylated byproduct **5-D**{R^1,-,*1*}. Further investigation (cleavage of resins **5**{R^1,R^2,*1*} was performed for various time periods and identical mixtures of compounds were obtained) showed that the side product was not formed during the cleavage of intermediates **5** from the resin but during the acylation of intermediates **4**{R^1,R^2} with *o*-nitrobenzoic acid.

In most of the tested cases, the dealkylation did not decrease the overall purity significantly and the intermediates **5**{R^1,R^2,R^3} were obtained in a sufficient purity ranging from 72 to 93% (UPLC-UV-traces). However, in the case of the intermediate **3**{*3*,*7*} prepared from chloroacetone, we obtained only a mixture of

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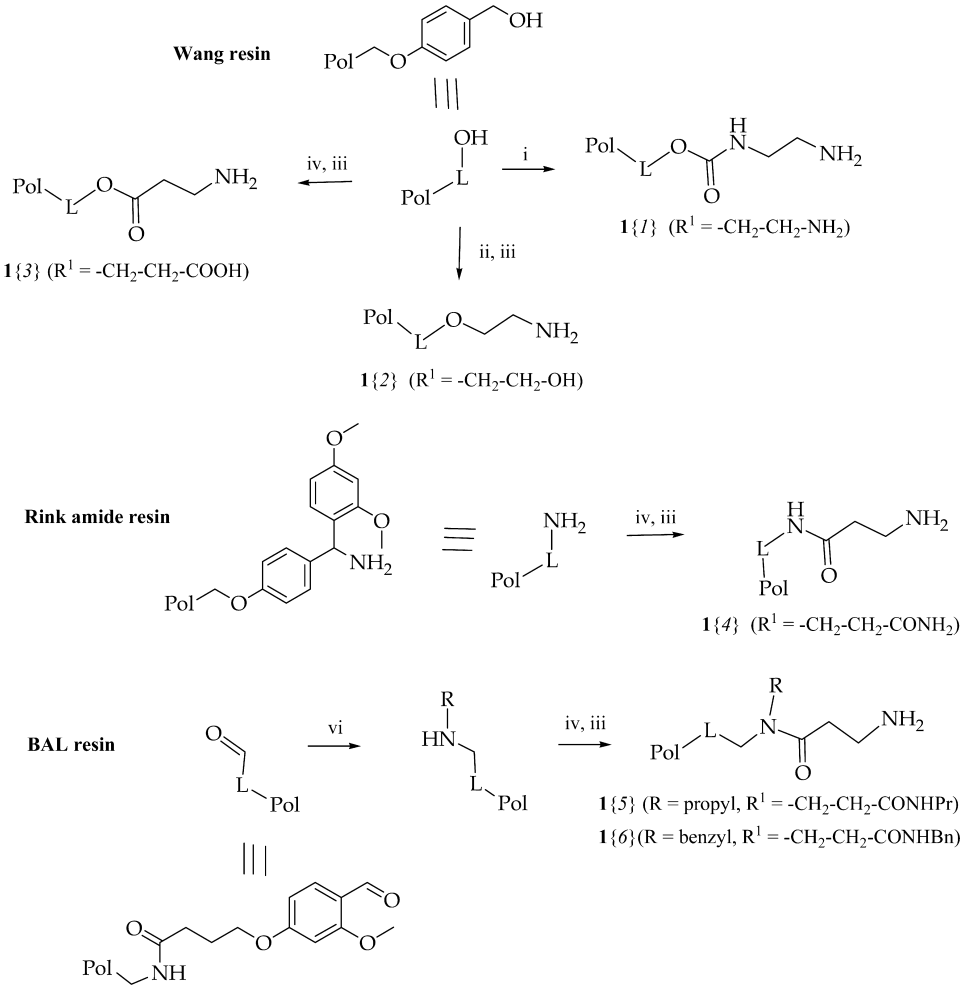
Scheme 2. Preparation of Immobilized Amines 1{R[1]}(for Resulting R[1] See Also Table 3)[a]



[a]Reagents: (i) (a) CDI, pyridine, DCM, rt, 3 h, (b) ethylenediamine, DCM, rt, 3 h; (ii) (a) trichloroacetonitrile, DBU, anhydrous DCM, rt, 1 h, (b) 2-(Fmoc-amino)ethanol, boron trifluoride diethyl etherate, anhydrous THF, rt, 30 min; (iii) 50% piperidine in DMF, rt, 10 min; (iv) Fmoc-β-Ala-OH, TPP, DIAD, anhydrous THF, rt, 5 h; (v) Fmoc-β-Ala-OH, HOBt, DIC, DMF, DCM, rt, 16 h; (vi) (a) amine, 10% acetic acid in anhydrous DMF, rt, 16 h, (b) sodium triacetoxyborohydride, 10% acetic acid in anhydrous DMF, rt, 4 h, (c) 20% piperidine in DMF, rt, 10 min.

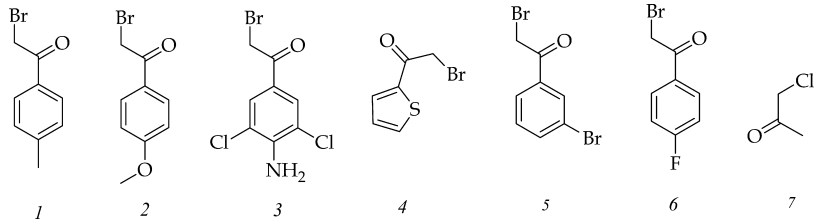


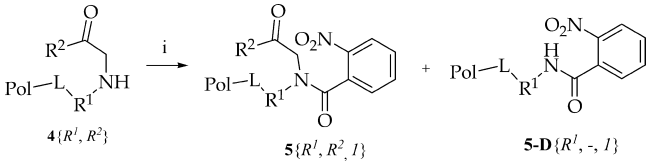
Figure 2. List of haloketones tested for R^2 substitution.

Table 1. Various Reaction Conditions for the Preparation of Intermediate 3{3,7}

reaction conditions	purity (%)[a]
DIEA, DMF, rt, 16 h	49
DIEA, DMF, rt, 16 h, repeated	70
DIEA, DMF, 40 °C, 16 h	75
DIEA, DMF, 40 °C, 16 h, repeated	46
proton sponge, DMF, rt, 16 h	70
DBU, DMF, rt, 16 h	mixture of compounds
DIEA, THF, rt, 16 h	mixture of compounds

[a]Calculated from HPLC-UV traces (PDA 200−600 nm) after cleavage from the polymer support.

Scheme 3. Dealkylation during Acylation with *o*-Nitrobenzoic Acid[a]



[a]Reagents: (i) *o*-nitrobenzoic acid, DIC, DMF, rt, 16 h.

compounds without the corresponding product. The use of intermediate prepared from 3-bromo-2-bromoacetophenone led

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to quantitative dealkylation, intermediate prepared from 4-fluoro-2-bromoacetophenone dealkylated from 60% so the both building blocks were excluded. To suppress the side reaction we tested the acylation of intermediate **4**{*1,1*} with alternative agents (such as isatoic anhydride or anthranilic acid) and species (HOBt ester, symmetrical anhydride) but the purity of intermediates **5** was still usually decreased (see Table 2). The best results were obtained with the use of a symmetrical anhydride prepared in situ from the corresponding *o*-nitrobenzoic acids in *N,N*-dimethylformamide.

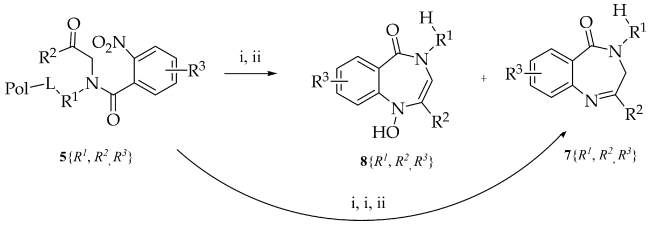
To introduce the third diversity position various *o*-nitrobenzoic acids were used (see Table 3) to acylate intermediates **4**{R^1,R^2}. After subsequent reduction of the nitrogroup and cleavage of the resulting material from the resin we did not detect the linear intermediates **6**{R^1,R^2,R^3} but only the final products **7**{R^1,R^2,R^3}. When the sample of the resin **6**{*1,1,1*} was treated with Fmoc-Cl and cleaved, the corresponding *N*-Fmoc intermediate was not detected which indicates the ring closure took place on-resin after reduction of the nitro group. After the reduction step, we detected the appearance of a side product in each case (10−30%, UPLC-UV traces). From UPLC-MS traces, we have concluded that the structure of the

Table 2. Summary of Alkylated/Dealkylated Product Ratio with Use of Intermediate 4{*3,1*} and Different Acylating Methods and Species

reagents	alkylated (%)[a]	dealkylated (%)[a]
o-nitrobenzoic acid, DIC, HOBt, DMF/DCM	64	30
o-nitrobenzoic acid, DIC, DMF	93	6
isatoic anhydride, DIPEA, DMF	0	21
anthranilic acid, DIC, HOBt, DMF/DCM	13	31
3-nitropthalic anhydride, DIEA, THF/dry	52	33
3-nitropyridine-2-carboxylic acid, DIC, DMF	85	2
4-bromo-2-nitrobenzoic acid, DIC, DMF	82	13
4-methyl-2-nitrobenzoic acid, DIC, DMF	72	20
5-methoxy-2-nitrobenzoic acid, DIC, DMF	87	12

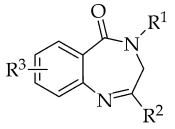
[a]Calculated from HPLC-UV traces (PDA 200−600 nm) after the cleavage from the polymer support.

Scheme 4. Side Product Formation after First-Round Reduction of Precursors 5[a]



[a]Reagents: (i) $SnCl_2 \cdot 2H_2O$, DIEA, deoxygenated DMF, rt, 16 h; (ii) 50% TFA in DCM, rt, 30 min.

Table 3. List of Final Compounds



7{R^1, R^2, R^3}

Compound	R^1	R^2	R^3	Purity (%)* (after C18 cartridge)	Purity (%)* (after semiprep. HPLC)	Yield (%)
7{*2,1,1*}	–OH		–H	97	99	30
7{*3,1,1*}	–COOH		–H	72	99	26
7{*3,2,1*}	–COOH	–OCH_3	–H	93	99	47
7{*3,3,1*}	–COOH	Cl, –NH_2, Cl	–H	88	99	33
7{*3,4,1*}	–COOH	S	–H	83	99	22
7{*3,1,2*}	–COOH		–8-Br	85	99	24
7{*3,1,3*}	–COOH		–7-OCH_3	73	99	14
7{*3,1,4*}	–COOH		–8-CH_3	87	99	15
7{*3,1,5*}	–COOH		Pyridine cycle	76	96	28
7{*4,1,1*}	–$CONH_2$		–H	79	96	30
7{*5,1,1*}	–CONHpropyl		–H	96	99	26
7{*6,1,1*}	–CONHbenzyl		–H	85	99	29
7{*1,1,1*}	–NH_2		–H	77	-	NI
7{*1,2,1*}	–NH_2	–OCH_3	–H	78	-	NI
7{*1,3,1*}	–NH_2	Cl, –NH_2	–H	63	-	NI
7{*1,4,1*}	–NH_2	Cl, S	–H	58	-	NI

*Calculated from HPLC-UV traces (PDA 200−600 nm), NI = not isolated due to the decomposition during semiprep. HPLC isolation.

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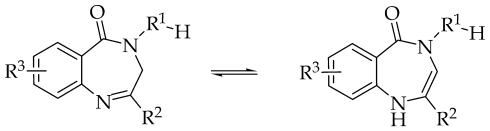
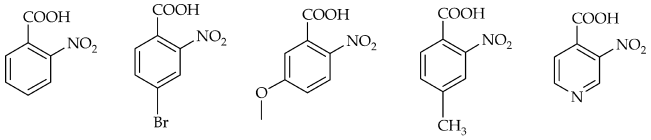


7A 7B

Figure 3. Two possible tautomeric forms of the final products.



1 *2* *3* *4* *5*

Figure 4. List of *o*-nitrobenzoic acids successfully used for the verification of the synthetic route.

side products corresponds to *N*-hydroxyderivatives **8**{R^1,R^2,R^3} formed after incomplete reduction of the nitrogroup to hydroxylamine derivative. After repeating the reduction step, the side products **8**{R^1,R^2,R^3} were not detected, which is in accordance with the theory of hydroxylamine intermediate formation.

The final compounds **7**{R^1,R^2,R^3} were generally obtained in very good crude purity (see Table 3), and their final purification was achieved by the use of flash chromatography on reversed phase C^{18} cartridges and subsequent reverse phase semipreparative HPLC. The use C^{18} cartridges was necessary to remove tin(II) and tin(IV) salts otherwise HPLC column was clogged during purification. During the isolation process, an unexpected instability of amino group containing derivatives **7**{*1*,R^2,R^3} was observed. Because of their decomposition, such substances have not been isolated in a pure form. The structure of the final products was confirmed with the help of ^{1}H and ^{13}C NMR spectrometry and HRMS.

We also investigated the tautomerism of prepared 1,4-benzodiazepine-5-ones since at least two possible tautomeric forms (7A) and (7B) have to be considered. We observed a broad singlet at around 4.20 ppm in the ^{1}H NMR spectra of the studied compounds which corresponds to a methylene group of tautomeric form (**7A**). The tautomers (7A) seem to be the only present form of the studied compounds under the experimental conditions used. We have proven this suggestion with the help of ^{1}H−^{1}H COSY and ^{1}H−^{13}C edited HSQC experiments in the case of compound **7**{*5*,*1*,*1*}. The formation of these kind of tautomers is in accordance with previously studied derivatives.[9,10]

In conclusion, we have developed a simple method for the preparation of novel trisubstituted 3,4-dihydro-benzo[e][1,4]-diazepin-5-ones with the use of solid-phase based synthesis. Various building blocks have been successfully tested and many others are commercially available. Final compounds were obtained in very good crude purity containing usually only one side product separable with the use of reverse phase chromatography. On the other hand, some limitations were observed: (i) the reaction pathway seems not to be well applicable for bromoketones substituted only with electron withdrawing ligands due to a formation of significant side-products, (ii) amino group containing ligands in position 4 of the benzodiazepinone scaffold exhibited unexpected unstability and decomposed during purification procedure. Despite this fact the described chemistry is still applicable for (semi)automated combinatorial synthesis of chemical library to produce novel derivatives for biological screening.

■ ASSOCIATED CONTENT

Supporting Information

Details of experimental synthetic and screening procedures and spectroscopic data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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