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Synthesis of an anionically substituted nitroindoline-caged GABA reagent that has reduced affinity for GABA receptors☆

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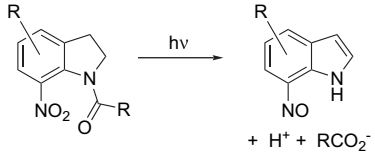
Abstract—A 7-nitroindolin-1-yl amide of the neuroactive amino acid γ-aminobutryate (GABA) has been synthesised with two phosphate groups attached to the indoline nucleus at 4-alkoxy substituent. The compound is designed to release GABA on a sub-microsecond time scale in response to flash photolysis with near-UV light. The presence of the high negatively charged substituent shows evidence that interaction of the GABA conjugate with ionotropic GABA receptors is much reduced in comparison with an earlier nitroindoline-GABA compound that had no charged groups on the indoline nucleus. In experiments to develop the eventual synthetic route, an unusual reductive cleavage of a TBDMS ether was observed as a significant side reaction during reduction of an indole to an indoline with $NaBH_3CN$ in glacial acetic acid. In the eventual synthetic route, the primary amine of GABA was masked as an azide until the final stage of the synthesis, which overcame significant problems with other forms of amine protection.

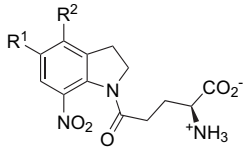
1. Introduction

Photolabile conjugates of bioeffector species, that are known as 'caged' compounds, are commonly used in biological applications for their ability to release an active bioeffector at or near its site of action in response to a brief pulse of near-UV light (typically in the ~300–350 nm range). Their purpose is to assist in time-resolved studies of biological processes and numerous reviews of their preparation and use are available.[1] As part of our studies of these reagents, we have previously described 7-nitroindoline-caged neuroactive amino acids, particularly the 5-(methoxycarbonyl)-methyl and 4-methoxy compounds **1** and **2**, that are able to release L-glutamate on a sub-microsecond time scale upon flash photolysis.[2–4] The overall reaction of these particular reagents upon irradiation in aqueous solution to release a carboxylate anion, accompanied by a nitrosoindole by-product, is shown in Scheme 1.

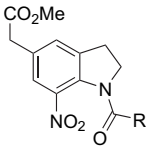


Scheme 1. Overall photocleavage reaction of 1-acyl-7-nitroindolines in aqueous solution.

Evaluation of the pharmacology of these reagents showed that the glutamate conjugates **1** and **2** did not bind to glutamatergic receptors,[5b] making them suitable as reagents to investigate synaptic function in neuronal cells, and these and other acyl nitroindolines have become established as good experimental tools for this purpose.[5]



1 $R^1 = CH_2CO_2Me$, $R^2 = H$
2 $R^1 = H$, $R^2 = OMe$



3 $R = (CH_2)_3NH_3^+$
4 $R = CH_2NH_3^+$

In contrast to these glutamate reagents, conjugates of γ-aminobutyrate (GABA) and glycine related to structure **1**, i.e., compounds **3** and **4**, were found to inhibit and slow the action of their respective ionotropic GABA or glycine receptors on neuronal cells and the response to photolytically released GABA or glycine was therefore blunted.[5b] We have sought a means to abolish or restrict these unwanted properties of the 7-nitroindoline conjugates of GABA and glycine, in order to make available reagents that could complement the successful glutamate derivatives **1** and **2** in electrophysiological research. The present paper is concerned with development of a GABA reagent, but the strategy employed may also be applicable to an analogous glycine compound.

It has been reported that the α-carboxy-2-nitrobenzyl ester of GABA, **5**, had no pharmacological effects,[6a] albeit

☆ The work described here is, in part, the subject of patent applications US Appl. 11/507961 and EP Appl. 06254350.4.

Keywords: Photolysis; Protecting groups; Reduction; Nitration.

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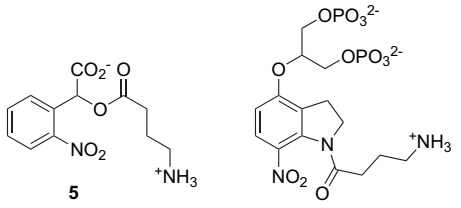
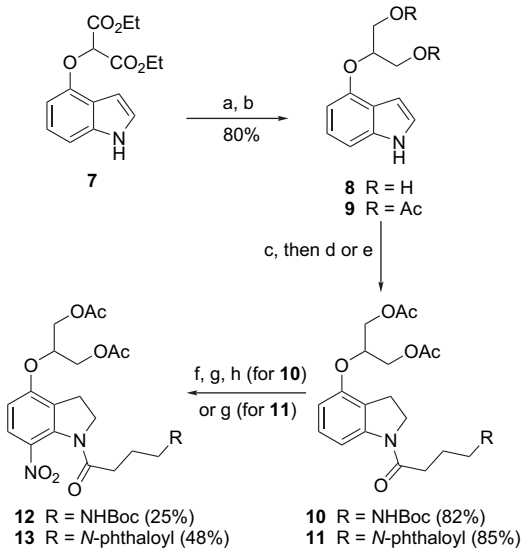
a more recent study indicated that GABA receptors in the dentate gyrus are inhibited by this compound.[6b] It is also generally recognised that esters of this type are susceptible to hydrolysis and are therefore prone to leak the bioeffector prior to photolysis. In contrast, nitroindoline compounds such as **1** and **2** are highly resistant to hydrolysis at neutral pH.[2] We hypothesised that the negative charge of the α-carboxylate group present in **5** was a key feature in terms of blocking binding of **5** to at least some classes of GABA receptors and therefore set out to prepare a nitroindoline derivative of GABA with a high concentration of negative charge. Our aim was to place this negative charge as close as feasible to the indoline nucleus.



Some initial efforts to place negatively charged groups at both the 3- and 4-positions of the indoline nucleus were bedevilled by synthetic problems and eventually our target species became the diphosphate **6**. It was anticipated that a successful outcome in terms of a lack of pre-photolysis pharmacology for this compound might also be beneficial for a glycine conjugate. The design of **6** incorporated a substituted variant of the 4-methoxy group that is present in **2**, since the latter is known to have beneficial effects on the efficiency of the photocleavage reaction.[3a] Compound **6** is densely functionalised and highly water-soluble, so its synthesis and purification presented a number of challenges, particularly with appropriate protection of the γ-amino group, that we had not encountered in previous studies of nitroindoline phototriggers. The synthetic permutations that were explored are briefly outlined here, both to give important background to our eventual development of an effective synthetic route and to report some unexpected results that may be of interest in other situations.

2. Results and discussion

Approaches to the synthesis of **6** began from the indoloxymalonate ester **7** (Scheme 2), for which we have previously described a convenient synthesis from commercial 4-hydroxyindole.[7] $LiAlH_4$ reduction of the diester gave the diol **8**, that was immediately protected as its crystalline diacetate **9**. Subsequent reduction to the indoline with $NaBH_3CN$ in acetic acid[8] and coupling with *N*-Boc-γ-aminobutyric acid gave the protected indolinyl-GABA derivative **10**. We had previously observed that nitration of the aromatic ring in a related Boc-protected glutamate derivative was accompanied by partial nitration/nitrosation of the Boc-protected amine,[3b] and later found that this could be prevented in the glutamate derivative by the use of double Boc protection of the amino side chain.[9] We therefore applied the same strategy to the GABA compound **10**, but in this case the second Boc group was evidently much more labile under the nitration conditions (claycop–Ac_2O in CCl_4)[3b] and failed to prevent unwanted nitration at the side-chain nitrogen. This greater lability of the second Boc group is probably a consequence of higher intrinsic basicity of the amino group in GABA compared to that in glutamate, and the product of the nitration reaction was a complex mixture from which the desired 7-nitro compound **12** was isolated in only 25% yield (data not shown).



Scheme 2. Reagents: (a) $LiAlH_4$; (b) Ac_2O–pyridine; (c) $NaBH_3CN$–AcOH; (d) EDC–*N*-Boc-GABA; (e) EDC–*N*-phthaloyl-GABA; (f) $(Boc)_2O$–Et_3N–DMAP; (g) claycop–Ac_2O–CCl_4; (h) 1 M TFA in CH_2Cl_2.

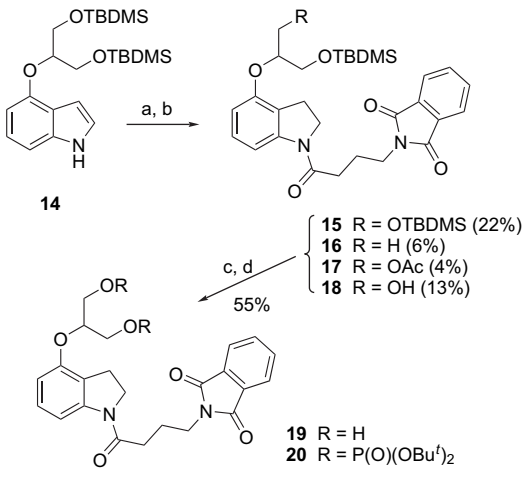
These difficulties with the Boc-protecting group led us instead to investigate phthaloyl protection of the primary amino group, and **11** was readily prepared, as shown in Scheme 2. Nitration was now more satisfactory, with the 7-nitro compound **13** being obtained in 48% yield (see Supplementary data). However it proved impossible to remove the acetate groups without substantial opening of the phthalimide ring. In retrospect this could have been anticipated, as alkaline cleavage of *N*-alkylphthalimides to the corresponding phthalamic acids is known to be rapid (for example, the half-life for ring opening of typical *N*-alkylphthalimides is ~0.3 s in 0.1 M NaOH at 30 °C).[10]

By this stage, it was evident that a more orthogonal protecting group strategy was needed, and we opted to retain the benefit of the phthaloyl protection but instead to use silyl protection for the diol. We expected to be able later to remove the silyl groups without risk to the integrity of the phthalimide. Thus **8** was protected as its bis-TBDMS ether **14** and reduced with $NaBH_3CN$ in acetic acid to the crude indoline, which was immediately coupled with *N*-phthaloyl-GABA. Surprisingly, the reaction mixture from this sequence was found to contain four products, of which the major component was the anticipated bis-TBDMS ether **15** (isolated yield ~30%, but contaminated by **16**—see below). Compounds of increasing polarity were isolated as pure materials and identified, respectively, as the mono-methyl compound **16** (6%), the acetate **17** (4%) and the alcohol **18** (13%).

We have been unable to find a precedent for the reduction of a primary alkyl silyl ether (or its parent alcohol) to the related methyl group, as in **16**, under such conditions, although

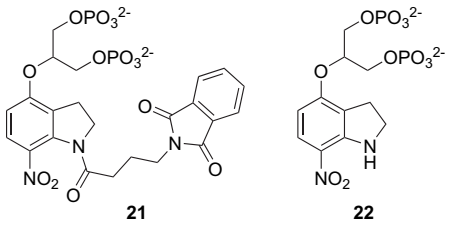
hydride reductions of benzylic alcohols under more strongly acidic conditions have been reported.[11] We hypothesise that steric congestion in the bis-TBDMS system of **14** promotes participation by the neighbouring aryloxy group, resulting in acid-catalysed formation of an oxiranium ion. The latter could undergo hydride reduction or displacement by acetate, in either case at the less-hindered primary carbon, to give the minor products **16** and **17**. It is unclear whether partial loss of one TBDMS group to give the mono-alcohol **18** occurred during the reaction or as an accidental consequence of the work-up procedure. Notably, no evidence of these side reactions had been seen during reduction of the acetate **9** under identical conditions, so steric crowding appears to be a significant contributor to this unusual reactivity.

Although compounds **15**–**18** could be separated, it was more efficient to treat the mixture first with buffered TBAF to remove all silyl groups and to isolate the crystalline diol **19**, free from contaminating mono-alcohols derived from **16** and **17**. Subsequent transformations of **19** that led to an initial synthesis of **6** in low yield are described here, since they provided important insights for the eventual more successful route that is described below, but the experimental details are reported only in the Supplementary data. The diol **19** was converted to the protected bis-phosphate **20** (Scheme 3), and this material was nitrated under homogeneous conditions ($Cu(NO_3)_2$–Ac_2O). Although we have used heterogeneous nitration with the claycop reagent extensively for other 1-acylindolines because of its advantageous regiochemical preference for 7-nitration,[3b] it was not effective in this case. It appears that the very bulky 4-substituent of **20** prevents entry of the indoline into the clay lattice and trials of claycop nitration here resulted in extensive recovery of starting material. Fortunately, homogeneous nitration was found to proceed with a strong preference for the 7-nitro isomer. Our accumulated experience from other indolines that the nature of the 4-alkoxy substituent can have substantial but idiosyncratic effects on the regioselectivity of nitration. Thus, maximising the yield of a required 7-nitro isomer can require some optimisation whenever a different 4-alkoxy substituent is present. Bulky substituents in the acyl group can also affect the efficiency of claycop nitration.



Scheme 3. Reagents: (a) $NaBH_3CN$–AcOH; (b) EDC–*N*-phthaloyl-GABA; (c) TBAF–THF–AcOH; (d) $Et_2NP(O^tBu)_2$-1*H*-tetrazole, then MCPBA.

Chromatography of the crude nitration product in the hope of separating the 5- and 7-nitro isomers resulted in loss of much material, and it seemed likely that partial deprotection of the phosphate groups had taken place during the nitration step. Nevertheless, the material isolated by chromatography was treated with TFA to achieve complete deprotection of the phosphate groups, and the water-soluble products were separated by reverse-phase HPLC to give a homogeneous material that was assigned as the 7-nitro bis-phosphate **21**. The structural assignment was initially on the basis of its UV–visible spectrum and, retrospectively, from its further transformation to the end product **6** for which full NMR characterisation was possible. The UV–visible and NMR spectra of 5- and 7-nitro-1-acyl-4-methoxyindolines have been described and readily allow the isomers to be distinguished.[3a] Preliminary experiments had already established that the very hydrophilic nature of **6** made it difficult or impossible to separate from its 5-nitro isomer by preparative reverse-phase HPLC, as it was too poorly retained to allow a practical separation to be achieved (although an analytical separation was possible with a high molarity mobile phase). Hence, we found it necessary to separate the 5- and 7-nitro isomers at the stage of compound **21**, in which the phthaloyl group provided a strong hydrophobic component to promote absorption on the HPLC column.



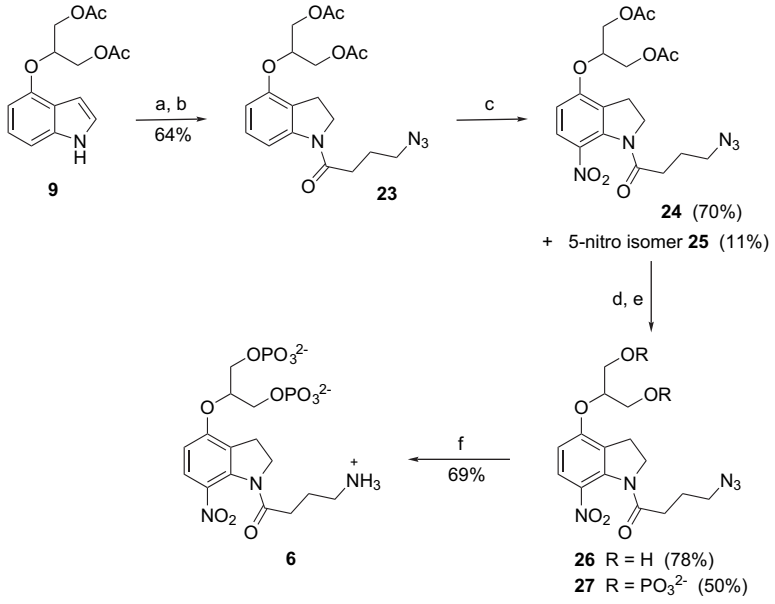
Completion of the synthesis only required removal of the phthaloyl protecting group but conventional hydrazinolysis was not applicable because of the relatively labile amide bond of the nitroindoline. This is exacerbated here by the unprotonated γ-amino group that would be present in the product under hydrazinolysis conditions and which can promote this cleavage by intramolecular attack to split off butyrolactam. Previous hydrolysis data for 1-acyl-7-nitroindolines have shown the effect of a γ-amino group.[2] A more appropriate procedure to remove the phthaloyl group was that of Ganem and co-workers, namely reduction of the phthalimide with borohydride in aqueous isopropanol, followed by mild acid hydrolysis.[12] Nevertheless, the strongly charged nature of **21** caused practical difficulties, since the compound as its sodium salt was insoluble in the preferred[12] solvent of 6:1 isopropanol–H_2O. Thus the sodium salt was converted to the tetrabutylammonium salt, which still required a sub-optimal 4:1 isopropanol–H_2O ratio to achieve adequate solubility. Borohydride treatment of this solution, followed by hydrolysis in aqueous acetic acid gave a mixture of **6** and the deacylated compound **22**. Separation of these two species was achieved by anion-exchange chromatography, that used a gradient of sodium acetate, pH 6 to maintain the amino group in its protonated form and **6** was subsequently recovered from the eluate by precipitation as its Ba^{2+} salt. Subsequent conversion to the soluble Na^+ salt gave the pure compound in a form suitable for biological

use, albeit in very poor yield. As mentioned above, analytical reverse-phase HPLC in a high molarity buffer can resolve **6** and its 5-nitro isomer, so it was easy to confirm that the material isolated from the above sequence was isomerically pure, and NMR and mass spectrometry confirmed the structural assignment.

Initial electrophysiological tests with **6** showed that current responses with a rise time of ~1 ms can be evoked by 1 ms flash photorelease of high GABA concentrations in hippocampal neurons.[13] This contrasts with the slow rise-times previously reported upon photolysis of **3** under similar conditions and indicates that the affinity of the reagent for GABA receptors in its unphotolysed form is much reduced from that of the earlier reagent **3**.[5b] Further testing to establish a full pharmacological characterisation will be reported elsewhere, but the promising initial data made it essential to develop a more satisfactory synthesis. It was clear from the experiences outlined above that the principal difficulty to be overcome in the synthesis of **6** lay in an acceptable means to protect the primary amino group, for which purpose neither Boc nor phthaloyl protection had been adequate. Furthermore, silyl protection of the hydroxyl groups on the 4-substituent was rather unsatisfactory in view of the unwanted side reactions described above during reduction of indole **14** to the corresponding indoline. To resolve the difficulties, we decided instead to carry the amino group through the synthesis as an azide, with the intention of releasing the free amine at a late stage, when it would no longer require protection. It seemed likely that the use of the azide as a protecting group would enable us to return to acetate protection of the hydroxyl groups on the 4-substituent, thereby avoiding side reactions during indole reduction that are described above. The route developed from this overall strategy is shown in Scheme 4.

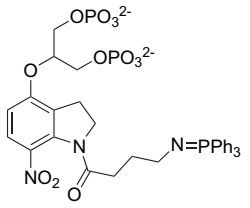
The indole **9** (Scheme 1) was reduced as before and the crude indoline was immediately coupled with 4-azidobutanoic acid to give the amide **23**. Homogeneous nitration, as for the previous compound **20**, proceeded with favourable regioselectivity to give the required 7-nitro isomer **24** in 70% isolated yield, together with the 5-nitro isomer **25** (11%). Separation of the two isomers was easily achieved by crystallisation of **24** from the isomeric mixture and mild alkaline hydrolysis then gave the diol **26** without significant cleavage of the relatively sensitive amide bond. Notably the synthetic route to this point avoids the problems encountered in the routes described above.

Diol **26** was poorly soluble in solvents such as acetonitrile or tetrahydrofuran, and phosphorylation by phosphoramidite chemistry, as described above for synthesis of **20**, was consistently unreliable. We turned instead to phosphorylation with phosphorus(V) reagents, of which pyrophosphoryl chloride has successfully been used for direct preparation of phosphate monoesters from a range of substrates, including diols of similar substructure to the 2-aryloxypropane-1,3-diol subunit present in **26**.[14] Treatment of a suspension of **26** in anhydrous ethyl acetate with excess pyrophosphoryl chloride smoothly gave the diphosphate **27** after aqueous quench. The aqueous solution contained substantial quantities of inorganic salts that made subsequent processing difficult, so the material was desalted by reverse-phase HPLC. This procedure was successfully achieved only by using a mobile phase with triethylammonium phosphate buffer, where ion-pairing with the lipophilic cation enabled the highly polar diphosphate **27** to be retained on the column while the excess inorganic salts were washed off. Elution with water alone then gave the product free of extraneous salts and the only remaining transformation required was reduction of the azido group to the corresponding amine. Staudinger reaction with triphenylphosphine was the method of choice to effect this without compromising the nitro group. Previous authors have described one-pot Staudinger reduction of azides to amines in THF containing a slight excess of the stoichiometric amount of water, the latter being



Scheme 4. Reagents: (a) $NaBH_3CN$–AcOH; (b) EDC–4-azidobutanoic acid; (c) $Cu(NO_3)_2$–Ac_2O–CH_2Cl_2; (d) NaOH–aq MeOH; (e) pyrophosphoryl chloride–EtOAc; (f) Ph_3P–aq DMF.

required for hydrolysis of the intermediate iminophosphorane,[15] while for other phosphorylated, highly water-soluble substrates the reaction has been conducted in a solvent of pyridine and aqueous ammonia.[16] The latter conditions were not appropriate because of the likely instability of the end product **6** with its amino group in the unprotonated form, as discussed above, while the presence of the two phosphate groups in **27** meant that the compound was not soluble in THF. The reduction was therefore conducted in DMF–H_2O (9:1) to achieve mutual solubility of all components. Surprisingly, the only reaction product under these conditions was not the expected amine **6** but was identified as the intermediate iminophosphorane **28**. The solution would presumably have been mildly acidic because of partial protonation of the phosphate groups of **27** after its isolation by preparative HPLC in pH 6 buffer, and the iminophosphorane was evidently stable under these conditions. In a preliminary experiment, **28** was isolated by preparative HPLC (also at pH 6) without any evidence of hydrolysis and was fully characterised by NMR and mass spectrometries (see Supplementary data), but in practice it was more convenient to hydrolyse the crude reduction product by incubation at pH 1 and ambient temperature, so releasing the end product **6**. The overall isolated yield of **6** from the reduction of **26** was ~67% after purification by anion-exchange chromatography, precipitation as its barium salt and reconversion to its sodium salt.



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The photochemical properties of **6** were assessed in two ways. Firstly, comparative irradiation of separate solutions of **6** and the established glutamate analogue **2** (at equal concentrations) was monitored by UV spectroscopy after increasing times of irradiation[3a] and showed identical spectroscopic changes with identical time courses for the two compounds (see Supplementary data). Thus the presence of the bulky, highly charged substituent at position 4 does not affect the photolysis and the quantum yield is the same (0.085) as previously reported for **2**.[3a] Photolysis was also quantitatively monitored by HPLC and released GABA was measured by amino acid analysis, as described for related compounds.[2,3a] GABA recovery was in the range 91–97%, i.e., quantitative within the experimental errors.

3. Conclusions

The final route described for synthesis of **6** is effective for preparation of the compound on a scale of a few hundred micromoles and involves no unwanted side reactions, apart from a small proportion of nitration at the 5-position during preparation of **24**. It has provided material on a sufficient scale for full pharmacological characterisation and these studies will be reported elsewhere. Meanwhile the synthesis of **6** has substantially broadened the scope of application of nitroindoline phototriggers, showing that it is possible to minimise adverse pharmacological properties that were evident in GABA conjugates of the first generation of these reagents. Steps are currently in hand to investigate whether the strategy employed here will also eliminate adverse pharmacology of the glycine conjugate **4**.

4. Experimental

4.1. General

^{1}H NMR spectra were determined on Varian Unityplus 500 or JEOL FX90Q spectrometers in $CDCl_3$ solution with TMS as internal reference, unless otherwise specified. Elemental analyses were carried out by MEDAC Ltd., Surrey, UK. Merck 9385 silica gel was used for flash chromatography. Analytical HPLC was performed on a 250×4 mm Merck Lichrospher RP8 column or a 125×4 mm Whatman Partisphere SAX column. Flow rates were 1.5 mL min^{-1} with either column. Preparative HPLC was carried out on a 2×30 cm column (Waters C_{18} packing, Cat. no. 20594) at 2 mL min^{-1} flow rate. Details of mobile phases are given at relevant points in the text. Preparative anion-exchange chromatography used a column of DEAE–cellulose (2×30 cm). Detection for all analytical and preparative work was at 254 nm. Organic solvents were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Hexane solvent (bp 40–60 °C) was redistilled before use. Triethylammonium bicarbonate solution was prepared by bubbling CO_2 into an ice-cold aqueous solution of 1 M triethylamine until the pH stabilised (pH ~7.4). Photolysis experiments were performed in a Rayonet RPR-100 photochemical reactor fitted with 16×350 nm lamps.

4.2. 4-(1,3-Diacetoxypropan-2-yloxy)indole (9)

$LiAlH_4$ (1.82 g, 48 mmol) was suspended in dry Et_2O (120 mL) in a 3-necked flask equipped with a reflux condenser and a pressure-equalising funnel, and cooled to 0 °C under nitrogen. A solution of diethyl indol-4-yloxymalonate **7** (3.49 g, 12 mmol; prepared as previously described[7]) in dry Et_2O (60 mL) was added dropwise. The cold bath was removed and the mixture was refluxed for 2 h, then cooled to 0 °C and water (1.82 mL) was added followed by 15% aq NaOH (1.82 mL) and finally by water (3×1.82 mL). The precipitated solid was filtered, washed thoroughly with EtOAc, and the combined washings were dried and evaporated to give crude 2-(1*H*-indol-4-yloxy)propane-1,3-diol **8** as a viscous oil (2.11 g). ^{1}H NMR (90 MHz) δ 9.64 (br s, 1H), 6.92–7.12 (m, 3H), 6.52–6.68 (m, 2H), 4.50 (quintet, J=5.4 Hz, 1H), 3.72–4.04 (m, 4H), 3.92 (br s, 2H). This material was dissolved in dry pyridine (25 mL) and treated with acetic anhydride (4.90 g, 48 mmol) and stirred at rt overnight. The solvent was evaporated and the residue was dissolved in EtOAc and washed successively with 1 M aq HCl, saturated aq $NaHCO_3$ and brine, dried and evaporated to a viscous oil. Trituration with ether gave **9** (2.79 g, 80%) as colourless crystals, mp 80–81 °C (EtOAc–hexanes). ^{1}H NMR (500 MHz) δ 8.21 (br s, 1H), 7.12 (dd, J=3.1, 2.4 Hz, 1H), 7.06–7.11 (m, 2H), 6.66 (dd, J=7.1, 1.1 Hz, 1H), 6.63–6.69 (m, 1H), 4.83 (quintet,

J=5.3 Hz, 1H), 4.44 (dd, J=11.8 and 5.6 Hz, 2H), 4.37 (dd, J=11.7 and 5.0 Hz, 2H), 2.07 (s, 6H). Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.96; H, 5.98; N, 4.73.

4.3. 1-[4-(*tert*-Butoxycarbonylamino)butanoyl]-4-(1,3-diacetoxypropan-2-yloxy)indoline (10)

To a solution of **9** (0.55 g, 1.9 mmol) in acetic acid (15 mL) was added portionwise $NaBH_3CN$ (0.47 g, 5.7 mmol), and the mixture was stirred at rt for 1 h. The solvent was evaporated and the residue was diluted with water, neutralised with $NaHCO_3$ and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give crude 4-(1,3-diacetoxypropan-2-yloxy)indoline (0.56 g, 100%) as a viscous oil. ^{1}H NMR (90 MHz) δ 6.95 (t, J=7.2 Hz, 1H), 6.24–6.68 (m, 2H), 4.62 (quintet, J=5.4 Hz, 1H), 4.20–4.46 (m, 4H), 3.64 (s, 1H), 4.52 (t, J=7.2 Hz, 2H), 2.94 (t, J=7.2 Hz, 2H), 2.06 (s, 6H). Without further purification, this material was dissolved in dry MeCN (20 mL) and treated with *N*-Boc-γ-aminobutyric acid (0.43 g, 2.1 mmol), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.52 g, 2.7 mmol) and the mixture was stirred at rt overnight. The solvent was evaporated and the residue was taken up in EtOAc, washed with dilute aq HCl, saturated aq $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–hexanes (3:2)] gave **10** (0.75 g, 82%) as a colourless viscous oil. ^{1}H NMR (500 MHz) δ 7.85 (d, J=8.1 Hz, 1H), 7.14 (t, J=8.1 Hz, 1H), 6.68 (d, J=8.1 Hz, 1H), 4.83 (br s, 1H), 4.69 (quintet, J=5.3 Hz, 1H), 4.27–4.38 (m, 4H), 4.05 (t, J=8.4 Hz, 2H), 3.16–3.23 (m, 2H), 3.10 (t, J=8.4 Hz, 2H), 2.46 (t, J=7 Hz, 2H), 2.07 (s, 6H), 1.92 (quintet, J=6.6 Hz, 2H), 1.42 (s, 9H). HRMS (FAB): calcd for $(C_{24}H_{34}N_2O_8+H)^+$ 479.2394, found 479.2393.

4.4. 4-[1,3-Di(*tert*-butyldimethylsilyloxy)propan-2-yloxy]indole (14)

Crude **8** (2.63 g), prepared as described in Section 4.2, was dissolved in dry CH_2Cl_2 (250 mL) and treated with imidazole (2.45 g, 36 mmol) and *tert*-butyldimethylsilyl chloride (4.52 g, 30 mmol) and the mixture was stirred at rt under nitrogen overnight. The precipitated colourless solid was filtered off and washed with CH_2Cl_2. The filtrate was washed successively with 0.5 M aq HCl, 0.5 M aq NaOH and brine, dried and evaporated to give **14** (4.13 g, 79%) as a colourless viscous oil. ^{1}H NMR (500 MHz) δ 8.11 (br s, 1H), 6.99–7.12 (m, 3H), 6.61–6.67 (m, 2H), 4.51 (quintet, J=5 Hz, 1H), 3.86–3.95 (m, 4H), 0.89 (s, 18H), 0.07 (s, 6H), 0.03 (s, 6H). HRMS (ES$^+$): calcd for $(C_{23}H_{41}NO_3Si_2+H)^+$ 436.2698, found 436.2718.

4.5. 1-(4-Phthalimidobutanoyl)-4-[1,3-di(*tert*-butyldimethylsilyloxy)propan-2-yloxy]indoline (15) and related compounds (16–18)

$NaBH_3CN$ (1.79 g, 28.5 mmol) was added portionwise to a solution of **14** (4.13 g, 9.5 mmol) in acetic acid (90 mL) and the mixture was stirred at rt for 1 h. The solvent was evaporated and the residue was diluted with water, neutralised with solid $NaHCO_3$ and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give crude 4-[1,3-di(*tert*-butyldimethylsilyloxy)propan-2-yloxy]indoline (3.14 g, 75%) as a viscous oil. ^{1}H NMR (90 MHz) δ 6.82 (t, J=7.2 Hz 1H), 6.04–6.32 (m, 2H), 4.16–4.32 (m, 1H), 3.64–3.88 (m, 4H), 3.46 (t, J=7.2 Hz, 2H), 2.88 (t, J=7.2 Hz, 2H), 2.04 (s, 1H), 0.88 (s, 18H), 0.04 (s, 12H). This material was dissolved in dry MeCN (95 mL) and treated with 4-phthalimidobutanoic acid (2.66 g, 11.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.55 g, 13.3 mmol) and the mixture was stirred at rt overnight. The solvent was evaporated and the residue was taken up in EtOAc, washed with 0.5 M aq HCl, saturated aq $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–hexanes (1:4)→(2:3)→(1:1)] gave four products. The first material eluted was **15** (1.69 g) as colourless crystals, mp 118–120 °C (MeOH). This material was estimated by ^{1}H NMR to contain ~20% of 1-(4-phthalimidobutanoyl]-4-[1-(*tert*-butyldimethylsilyloxy)propan-2-yloxy]indoline **16** (see below) and was not characterised further.

The second material eluted was 1-(4-phthalimidobutanoyl)-4-[1-(*tert*-butyldimethylsilyloxy)propan-2-yloxy]indoline **16** (0.22 g, 6%) as colourless crystals, mp 94–96 °C (Et_2O–hexanes). ^{1}H NMR (500 MHz) δ 7.81 (dd, J=5.4 and 2.9 Hz, 2H), 7.71 (d, J=8.2 Hz, 1H), 7.68 (dd, J=5.4 and 2.9 Hz, 2H), 7.07 (t, J=8.2 Hz, 1H), 6.51 (d, J=8.2 Hz, 1H), 4.13–4.19 (m, 1H), 4.02 (t, J=8.5 Hz, 2H), 3.74–3.92 (m, 6H), 3.10 (t, J=8.5 Hz, 2H), 2.47 (t, J=7.1 Hz, 2H), 2.15 (quintet, J=7.1 Hz, 2H), 1.23 (d, J=6.2 Hz, 3H), 0.90 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H). LRMS (ES$^+$): calcd for $(C_{29}H_{38}N_2O_5Si+H)^+$ 523, found 523. Anal. Calcd for $C_{29}H_{38}N_2O_5Si+H_2O$: C, 64.64; H, 7.45; N, 5.18. Found: C, 64.70; H, 7.13; N, 5.12.

The third material eluted was 1-(4-phthalimidobutanoyl)-4-[1-acetoxy-3-(*tert*-butyldimethylsilyloxy)propan-2-yloxy]indoline **17** (0.15 g, 4%) as colourless viscous oil. ^{1}H NMR (500 MHz) δ 7.81 (dd, J=5.4 and 2.9 Hz, 2H), 7.67–7.74 (m, 3H), 7.07 (t, J=8.2 Hz, 1H), 6.63 (d, J=8.2 Hz, 1H), 4.52 (quintet, J=5.2 Hz, 1H), 4.28–4.36 (m, 2H), 4.01 (t, J=8.5 Hz, 2H), 3.77–3.85 (m, 4H), 3.08 (t, J=8.5 Hz, 2H), 2.47 (t, J=7.1 Hz, 2H), 2.15 (quintet, J=7.1 Hz, 2H), 2.04 (s, 3H), 0.90 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). HRMS (ES$^+$): calcd for $(C_{31}H_{40}N_2O_7Si+H)^+$ 581.2678, found 581.2668.

The fourth material eluted was 1-(4-phthalimidobutanoyl)-4-[1-(*tert*-butyldimethylsilyloxy)-3-hydroxypropan-2-yloxy]-indoline **18** (0.48 g, 13%) as colourless crystals, mp 74–76 °C (EtOAc–hexanes). ^{1}H NMR (500 MHz) δ 7.82 (dd, J=5.4 and 2.9 Hz, 2H), 7.73 (d, J=8.2 Hz, 1H), 7.68 (dd, J=5.4 and 2.9 Hz, 2H), 7.07 (t, J=8.2 Hz, 1H), 6.62 (d, J=8.2 Hz, 1H), 4.41 (quintet, J=5 Hz, 1H), 4.03 (t, J=8.5 Hz, 2H), 3.80–3.95 (m, 6H), 3.10 (t, J=8.5 Hz, 2H), 2.48 (t, J=7.1 Hz, 2H), 2.10–2.19 (m, 3H), 0.90 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H). Anal. Calcd for $C_{29}H_{38}N_2O_6Si$: C, 64.66; H, 7.11; N, 5.20. Found: C, 64.44; H, 7.10; N, 5.06.

4.6. 1-(4-Azidobutanoyl)-4-(1,3-diacetoxypropan-2-yloxy)indoline (23)

Crude **8** (1.47 g, 5 mmol), prepared as described in Section 4.2, was dissolved in dry MeCN (40 mL) and treated with

4-azidobutanoic acid[17] (0.78 g, 6 mmol), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.34 g, 7 mmol) and the mixture was stirred at rt overnight. The solvent was evaporated and the residue was dissolved in EtOAc (50 mL) and washed with dilute aq HCl, saturated aq $NaHCO_3$ and brine, dried and evaporated. Flash chromatography [EtOAc–hexanes (2:3)] gave **23** (1.33 g, 64%) as colourless crystals, mp 61–63 °C (Et_2O–hexanes). ^1H NMR (500 MHz) δ 7.87 (d, J=7.9 Hz, 1H), 7.16 (t, J=8.2 Hz, 1H), 6.68 (d, J=8.3 Hz, 1H), 4.70 (quintet, J=5.3 Hz, 1H), 4.34 (dd, J=11.9 and 5.7 Hz, 2H), 4.29 (dd, J=11.9 and 4.8 Hz, 2H), 4.07 (t, J=8.5 Hz, 2H), 3.45 (t, J=6.4 Hz, 2H), 3.12 (t, J=8.4 Hz, 2H), 2.51 (t, J=6.9 Hz, 2H), 2.06 (s, 6H), 2.03 (quintet, J=6.6 Hz, 2H). Anal. Calcd for $C_{19}H_{24}N_4O_6$: C, 56.43; H, 5.98; N, 13.85. Found: C, 56.23; H, 5.98; N, 13.61.

4.7. 1-(4-Azidobutanoyl)-4-(1,3-diacetoxypropan-2-yloxy)-7-nitroindoline (24) and its 5-nitro isomer (25)

Copper nitrate hemipentahydrate (0.64 g, 2.75 mmol) was added to a solution of **23** (1.02 g, 2.5 mmol) in a mixture of CH_2Cl_2 (25 mL) and acetic anhydride (50 mL) and the solution was stirred at rt. The reaction progress was monitored by TLC [EtOAc–hexanes (1:1)] and after 25 h the solution was concentrated. The residue was re-evaporated from toluene and diluted with EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ and brine, dried and evaporated to a brown viscous oil. Flash chromatography [EtOAc–hexanes (1:1)] and crystallisation (EtOAc–hexanes) gave **24** (0.78 g, 70%) as yellow crystals, mp 101–102 °C. UV: λ_{max} (EtOH)/nm (ε/M^{-1} cm^{-1}) 249 (22 280), 299 (5270), 340sh (4040); λ_{max} [EtOH–25 mM Na phosphate, pH 7.0 (1:24)]/nm (ε/M^{-1} cm^{-1}) 247 (19 750), 330 (4800). ^1H NMR (500 MHz) δ 7.74 (d, J=9.1 Hz, 1H), 6.79 (d, J=9.1 Hz, 1H), 4.80 (quintet, J=5.3 Hz, 1H), 4.35 (dd, J=12.0 and 5.8 Hz, 2H), 4.30 (dd, J=12.0 and 4.7 Hz, 2H), 4.24 (t, J=8.1 Hz, 2H), 3.44 (t, J=6.4 Hz, 2H), 3.10 (t, J=8.1 Hz, 2H), 2.58 (t, J=6.8 Hz, 2H), 2.07 (s, 6H), 2.02 (quintet, J=6.4 Hz, 2H). Anal. Calcd for $C_{19}H_{23}N_5O_8$: C, 50.78; H, 5.16; N, 15.58. Found: C, 50.86; H, 5.21; N, 15.64.

The residue from the mother liquor was re-chromatographed to give 1-(4-azidobutanoyl)-4-(1,3-diacetoxypropan-2-yloxy)-5-nitroindoline **25** (0.12 g, 11%) as a pale viscous oil. ^1H NMR (500 MHz) δ 8.03–8.07 (m, 1H), 7.91 (d, J=8.2 Hz, 1H), 4.54 (quintet, J=5.1 Hz, 1H), 4.32 (d, J=5.0 Hz, 4H), 4.18 (t, J=8.6 Hz, 2H), 3.47 (t, J=6.3 Hz, 2H), 3.26 (t, J=8.6 Hz, 2H), 2.56 (t, J=7.2 Hz, 2H), 2.03 (quintet, J=6.4 Hz, 2H), 2.02 (s, 6H). This material was not characterised further.

4.8. 1-(4-Azidobutanoyl)-4-(1,3-dihydroxypropan-2-yloxy)-7-nitroindoline (26)

A solution of **24** (0.76 g, 1.7 mmol) in MeOH (85 mL), water (8.5 mL) and 1 M aq NaOH (4.1 mL, 4.1 mmol) was stirred at rt for 2 min, quenched with 1 M citric acid (8.5 mL) and concentrated to ~20 mL. The solution was diluted with water (30 mL) and washed with EtOAc (3×50 mL), and the combined organic extract was washed with saturated aq $NaHCO_3$ and brine, dried and evaporated to a brown viscous oil. Trituration with Et_2O gave **26** (0.49 g, 78%) as yellow needles, mp 78–79 °C (EtOAc–hexanes). ^1H NMR (500 MHz) δ 7.70 (d, J=9.2 Hz, 1H), 6.76 (d, J=9.2 Hz, 1H), 4.57 (quintet, J=4.8 Hz, 1H), 4.25 (t, J=8.1 Hz, 2H), 3.89–3.97 (m, 4H), 3.43 (t, J=6.4 Hz, 2H), 3.14 (t, J=8.0 Hz, 2H), 2.57 (t, J=6.9 Hz, 2H), 2.02 (t, J=7.6 Hz, 2H), 2.00 (quintet, J=6.4 Hz, 2H). HRMS (ESI): calcd for $(C_{15}H_{19}N_5O_6+H)^+$ 366.1408, found 366.1427.

4.9. 1-(4-Azidobutanoyl)-4-[1,3-bis(dihydroxyphosphoryloxy)propan-2-yloxy]-7-nitroindoline (27)

Pyrophosphoryl chloride (1.81 g, 7.2 mmol) was added at 0 °C to a suspension of the diol **26** (146 mg, 0.4 mmol) in anhydrous EtOAc (8.8 mL) and the mixture was stirred at 0 °C for 3 h. The reaction was quenched by the addition of ice-cold water (60 mL), adjusted to pH 6.6 with cold 1 M triethylammonium bicarbonate (~60 mL), and washed with Et_2O (3×50 mL). The aqueous phase was concentrated under reduced pressure to ~50 mL and the pH was raised to 7.0 by careful addition of Et_3N. The neutral solution was passed through a 0.2 μm membrane filter and analysed by anion-exchange HPLC [mobile phase; 100 mM Na phosphate, pH 6.0–20% MeCN (5:1), t_R 5.2 min] and by reverse-phase HPLC [mobile phase; 25 mM Na phosphate, pH 6.0–MeCN (25:1), t_R 13.8 min]. The solution was then loaded onto the preparative HPLC column (2 mL min^{-1}) that was pre-equilibrated with 25 mM triethylammonium phosphate, pH 6.0. The column was first washed with 25 mM triethylammonium phosphate, pH 6.0 for 2 h and then with water. The product was eluted after ~45 min when the conductivity of the eluent was reduced to that of water. Fractions were analysed by anion-exchange HPLC as above, and those containing the product were combined (154 mL) and quantified by UV–vis spectroscopy (1.28 mM, 198 μmol). The solution was then concentrated to ~15 mL, passed again through a 0.2 μm membrane filter and lyophilised to a pale yellow solid. The solid was dissolved in water (2 mL) and an aliquot (50 μL, 5 μmol) was exchanged to the Na^+ salt (Dowex 50, Na^+ form). ^1H NMR (500 MHz, D_2O, acetone ref.) δ 7.82 (d, J=9.0 Hz, 1H), 7.12 (d, J=9.3 Hz, 1H), 4.90 (quintet, J=5.5 Hz, 1H), 4.35 (t, J=7.8 Hz, 2H), 4.07–4.16 (m, 4H), 3.43 (t, J=6.8 Hz, 2H), 3.22 (t, J=7.9 Hz, 2H), 2.72 (t, J=6.2 Hz, 2H), 1.96 (quintet, J=6.4 Hz, 2H). This material was used without further characterisation.

4.10. 1-(4-Aminobutanoyl)-4-[1,3-bis(dihydroxyphosphoryloxy)propan-2-yloxy]-7-nitroindoline (6)

A solution of **27** (193 μmol) in water (2 mL) was diluted with DMF (18 mL) and treated with triphenylphosphine (355 mg, 1.35 mmol) and the mixture was stirred at rt under nitrogen for 20 h. The solution was concentrated under reduced pressure and the residue was diluted with water (60 mL). The solution (pH 3.1) was washed with EtOAc (3×50 mL) and the aqueous phase was separated and adjusted to pH 1.0 with 1 M aq HCl and stirred overnight at rt. Reverse-phase HPLC analysis as in Section 4.9 confirmed that all the azide was reduced, and analysis by anion-exchange HPLC as in Section 4.9 confirmed complete hydrolysis of iminophosphorane **28** (t_R 3.0 min, see Supplementary data). The solution was adjusted to pH 6.0 with 1 M

aq NaOH, diluted with water to 1350 mL (conductivity 2.6 mS cm^{-1}) and chromatographed on the DEAE-cellulose anion-exchange column, using a linear gradient formed from 10 and 550 mM NaOAc, pH 6.0 (each 1000 mL). Fractions containing the product, which eluted at ~300 mM NaOAc, were analysed as above, combined (255 mL) and quantified by UV–vis spectroscopy (0.597 mM, 152 μmol). The solution was concentrated to ~10 mL, diluted with water to 16 mL and treated with 2 M $Ba(OAc)_2$ (4 mL) and EtOH (8 mL) and allowed to stand at 4 °C overnight. The mixture was centrifuged and the supernatant was analysed by UV–vis spectroscopy (10% of original concentration, i.e., 90% precipitation). The precipitate was washed with water–EtOH (1:1) (5×20 mL) by resuspension and subsequent centrifugation after each wash cycle. The final precipitate was dissolved in water (30 mL) and mixed with Dowex 50 (Na^+ form; 5 g) for 2 h. The resin was filtered off, washed with water (20 mL) and the combined filtrates were adjusted from pH 8.15 to 7.0 with 1 M aq HCl. The filtrate was passed through a 0.2 μm membrane filter, lyophilised and the residue was dissolved in water (4 mL) and quantified by UV–vis spectroscopy to give pure **6** (33.15 mM, 133 μmol) as the Na^+ salt. ^1H NMR (500 MHz, D_2O, acetone ref.) δ 7.82 (d, J=9.3 Hz, 1H), 7.15 (d, J=9.3 Hz, 1H), 4.89 (quintet, J=4.5 Hz, 1H), 4.33 (t, J=8.0 Hz, 2H), 4.03–4.12 (m, 4H), 3.23 (t, J=8.0 Hz, 2H), 3.07 (t, J=7.6 Hz, 2H), 2.75 (t, J=6.4 Hz, 2H), 2.02 (quintet, J=6.4 Hz, 2H). LRMS (ESI): calcd for $(C_{15}H_{20}N_3O_{12}P_2+2H)^-$ 498.1, found: 498.2.

4.11. Quantitative photolysis and product analysis for (6)

Separate solutions of **6** (0.55 mM in 25 mM Na phosphate, pH 7.0 containing 5 mM dithiothreitol) were irradiated for varying times (25 or 45 s) in 1 mm path length cells (Rayonet Photochemical Reactor). The solutions were analysed by anion-exchange HPLC (mobile phase as in Section 4.9) and the extent of photolysis of each solution was determined by comparison of peak heights with those of non-irradiated controls. Aliquots of the photolysed solutions were also subjected to quantitative amino acid analysis. Measured GABA concentrations were 91–97% of the expected values from the extent of photolysis and were not affected by the concentration of dithiothreitol. The stock solution (22.5 mM) was also subjected to quantitative amino acid analysis and the measured free GABA contamination was 0.03%.

In a further experiment, separate solutions of **2** and **6** (each 0.46 mM) in 25 mM Na phosphate, pH 7.0 were irradiated for increasing times (0–180 s) in 1 mm path length cells and the extent of photolysis for each compound was monitored after each irradiation interval by UV–vis spectroscopy.

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Supplementary data

Experimental details for compounds **11**, **13**, **19**, **20**, **28** and initial preparation of **6**, together with analytical HPLC separation conditions for **6** and its 5-nitro isomer are given in the Supplementary data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.07.030.

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