Metal-Free [2+2]-Photocycloaddition of (Z)-4-Aryliden-5(4*H*)-Oxazolones as Straightforward Synthesis of 1,3-Diaminotruxillic Acid Precursors. Synthetic Scope and Mechanistic Studies

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ABSTRACT. The direct [2+2]-photocycloaddition of (*Z*)-2-phenyl-4-arylidene-5(4*H*)-oxazolones **1** to give 1,3-diaminotruxillic cyclobutane-derivatives **2** in very good yields (75-100%) is reported. The reaction takes place by irradiation of CH₂Cl₂ solutions of **1** with the blue light (465 nm) provided by LED lamps of low power (around 1 W) for 72 h. Four isomers of the 1,3-diaminotruxillic cyclobutanes **2** were obtained, all of them fully characterized by a combination of NMR spectroscopy and X-ray diffraction analysis. The reaction shows a certain selectivity, since one of the isomers (the epsilon) is obtained preferentially, and works for electron-releasing and electron-withdrawing substituents at the arylidene ring. A novel setup is presented for the in-line monitoring of the continuous flow photo-assisted synthesis of the cyclobutane derivatives **2** by NMR spectroscopy, with the microreactor dramatically reducing reaction times to only 30 minutes with clear product distribution of up to four isomers. The mechanism of this [2+2]-photocycloaddition has been calculated by DFT methods, explaining all experimental findings. The reaction takes place through a stepwise formation of two new C-C bonds through a transient diradical singlet intermediate. The isomeric distribution of the final products is not due to equilibration processes, but instead reflects the kinetic preference during the rate limiting C-C bond formation step.

INTRODUCTION. The cyclobutane ring is a structural motif often found in molecular compounds, natural or synthetic, which show well-established biological or pharmacological activity.¹⁻⁴ Truxillic acids (Figure 1a) are tetra-substituted cyclobutanes, which are very well-known because of their antinociceptive and antimuscarinic activities, and also due to their relationship with the glucose metabolism.⁵⁻¹¹ In particular, the 1,3-diaminotruxillic acid derivatives (Figure 1b shows all possible stereoisomers) are object of active research from recent years because they behave as Glucagon-Like-Peptide 1 (GLP 1) agonists in the treatment of diabetes mellitus type 2.¹²⁻¹⁸ Therefore, this is an interesting family of compounds due to their recognized properties in different pharmacological targets.

Figure 1. Schematic representation of (a) truxillic acids, showing the basic cyclobutane skeleton, and (b) the different isomers of 1,3-diaminotruxillic acids, showing the cyclobutane ring and the relative arrangement of the substituents

We have recently reported the selective synthesis of 1,3-diaminotruxillic derivatives in three steps starting from readily available (*Z*)-2-aryl-4-arylidene-5(4*H*)-oxazolones, following the method shown in Figure 2.^{19,20} The first step is the *ortho*-palladation of the oxazolone through

regioselective C-H bond activation at the 4-arylidene ring and formation of clamshell dimeric structures, supported by carboxylate bridges (step a). In these structures the exocyclic C=C bonds of the oxazolones are placed face-to-face in close proximity, in an optimal arrangement to undergo a [2+2]-photocycloaddition. The second step is, obviously, the irradiation of the *ortho*-palladated dimers using LED devices with selected wavelengths. The combination of the geometrical constraints and the optimal wavelengths result in the stereoselective [2+2]-photocycloaddition of the C=C bonds and formation of the corresponding dimers containing the cyclobutanes as single isomers (step b). The third step is the hydrogenation in methanol of the cyclobutane-containing dimers, which occur with reduction of the *ortho*-palladated complexes to Pd(0) and release of the cyclobutanes. It is worthy to note that, because the hydrogenation takes place in methanol, the oxazolone ring opens in this step through methanolysis (step c).^{19,20}

Figure 2. Pd-templated assisted three step-synthesis of 1,3-diaminotruxillic derivatives: (a) orthopalladation and incorporation of the Pd to the oxazolone skeleton, (b) [2+2]-photocycloaddition with formation of the cyclobutane ring, and (iii) hydrogenation and liberation of the 1,3-diaminotruxillic methyl ester as key steps.

This method offers a series of undeniable advantages: (a) the scope of the reaction is quite general and tolerates different substituents at the 2-aryl as well as the 4-arylidene rings; (b) the process is fully stereoselective, due to the template effect of the clamshell structure imposed by the $\text{Pd}_2(\mu\text{-O}_2\text{CR})_2$ moiety, and only the epsilon stereoisomer of the 1,3-diaminotruxillic derivative is obtained (see Figures 1 and 2); (c) the reaction is very efficient because it takes place in few minutes using low amounts of energy; (d) the reaction can be further accelerated using microreactors in continuous flow, this fact also allowing the scaling to the preparation of large amounts of compounds. However, even taking into account all these advantages, it is easily noticeable that there is room to further improve this interesting reaction: (a) it is a multi-step procedure which uses stoichiometric amounts of expensive palladium salts; (b) some photocycloadditions are reversible, and this fact cannot be controlled by optimization of the reaction conditions; (c) the hydrogenation of some substrates produces modifications in some functional groups or gives side-products.

It is easy to see that the best scenario would be that in which the straight [2+2]-photocycloaddition of the 4-arylidene-5(4*H*)-oxazolone takes place, trying to keep the advantages of selectivity and efficiency provided by the palladium center. However, the reports found in the literature for the studies on the irradiation of oxazolones attempting a simple [2+2]-photocycloaddition, shown in Figure 3, are quite discouraging. The first contribution of Mohr *et al* (Figure 3a) reported that oxazolones were able to give a [2+2]-cycloaddition.²¹ Surprisingly, the reaction took place by addition through the C=N bonds, affording diazetidines, instead of the usual process through the C=C bonds.²¹ This reaction fallen in the oblivion until 2012, when Wang and coworkers described some few examples of photocycloaddition of oxazolones (Figure 3b), this time through the C=C bonds. However, the reported cases took place with low efficiency, because

four isomers with global yields as low as 10% were obtained in all cases and, to achieve these low conversions, the reaction needed a high-power irradiating source (500W) and long reaction times (more than 3 days).¹⁵ Moreover, the method was not valid for a wide range of oxazolones. Almost at the same time Sampedro *et al* described that, using ultraviolet radiation, the only observable process for *Z*-5(4*H*)-oxazolones in solution is the *Z* to *E* isomerization (Figure 3c, left part).²²⁻²⁴ Very recently, the same group noticed the dimerization of 5(4*H*)-oxazolones in solid state (Figure 3c, right part), in just a single case where the topochemical conditions of intermolecular C-C distances shorter than 4.2 Å (Schmidt criterion) are accomplished.²⁵ Therefore, sparse results have been obtained previously in this area, since each attempted irradiation has given a different result. It is clear that the synthesis of 1,3-diaminotruxillic acid derivatives through photocycloaddition of 5(4*H*)-oxazolones still lacks of a general method, suitable for different substituents and affording the corresponding products in good yields, under mild conditions and short reaction times.

In this contribution we report the highly efficient [2+2]-photocycloaddition of different 2-phenyl-4-(het)arylidene-5(4*H*)-oxazolones **1a-i**, in CH₂Cl₂ solution, by irradiation with the blue light (465 nm) provided by low-power LEDs. In this way, the 3,10-dioxa-1,8-diazadispiro[4.1.4⁷.1⁵]dodeca-1,8-diene-4,11-diones **2a-i**, precursors of 1,3-diaminotruxillic acids, can be obtained in a single step in conversions up to 100%. A novel setup for the in-line NMR monitorization of the on-flow [2+2]-photocycloaddition of diverse 2-phenyl-4-(het)arylidene-5(4*H*)-oxazolones **1b-d** in CH₂Cl₂ is presented. This setup enables the in-line, remote monitoring of the formation of up to four isomers, with the enhancement of the reaction conversion by microchannels of high surface to volume ratio. Moreover, we have determined the mechanism of the photocycloaddition by molecular modelling, using DFT methods. The reaction takes place

through a stepwise formation of the two new C-C bonds through an unexpected transient diradical singlet intermediate.

Figure 3. Previous work found in the literature concerning the synthesis of 1,3-diaminotruxillic derivatives and comparison with the present contribution.

RESULTS AND DISCUSSION

1.- Synthesis and Characterization of 1,3-diaminotruxillic precursors. The *Z*-oxazolones **1a-1i** have been prepared following published methods (see Supporting Information, SI). We have selected the examples shown in Table 1 and Figure 4 aiming to cover all possible situations: 4-arylidene and 4-hetarylidene (thiophene) rings, with electron-releasing (OMe, Me) or electron-withdrawing (NO₂) substituents, located at *ortho*, *meta* and/or *para* positions. The solutions of the oxazolones **1a-1i** in CH₂Cl₂ were irradiated (while stirred) using the blue light (465 nm) provided by 20 LEDs supported on a printed circuit board (PCB), which gives a maximum power of 1W. The device used for irradiation (SI), is equipped with an effective air circulation system, to eliminate the heat dissipation from the LEDs. The cyclobutanes **2a-2i** were obtained as a result of the irradiation, as shown in Figure 4 and Table 1.

Figure 4. Synthesis of 1,3-diaminotruxillic precursors **2a-2i** by photocycloaddition of oxazolones **1a-1i**, promoted by blue light

Table 1. Conversion (%) of oxazolones **1a-i** into 1,3-diaminotruxillic derivatives **2a-2i**, detected isomers and relative amount (%) of each diaminotruxillic derivative **2**. The reaction was performed in batch conditions, irradiating a solution of **1a-i** in CD₂Cl₂ at 465 nm for 72 h

	epsilon 2(a-i)1	alpha 2(a-i)2	epi 2(a-i)3	gamma 2(a-i)4
(1a)	37.1	8.7	2.6	n.d. ^a 51.5 ^b
(1b)	68.0	18.0	9.8	4.2
(1c)	43.7	27.2	17.0	12.1
(1d)	67.3	23.9	5.5	3.3
(1e)	76.5	13.7	6.5	3.3
(1f)	49.0	27.3	14.0	9.7
(1g)	88.0	7.6	3.1	1.3
(1h)	75.0	n.d. ^a	n.d. ^a	25.0 ^c
(1i)	37.8	26.1	23.8	12.3

a) n.d. = not detected; (b) conversion of 48.5% in the best case, 51.5% unreacted **1a**; (c) conversion of 75%, 25% unreacted **1h**.

In general, the initial yellow color of the oxazolones faded during the irradiation process, due to the loss of conjugation produced during the formation of the cyclobutanes. The optimization of the reaction time was performed by monitoring the reaction by ^1H NMR, extracting an aliquot of the reaction mixture and replacing the CH_2Cl_2 by CD_2Cl_2 . This shows the progressive disappearance of the signal due to the olefinic protons in **1a-1i** (located in the 7.1-7.9 ppm range) and the clear growth of a set of new peaks in the 4-5 ppm region, this upfield shift is in agreement with the formation of the cyclobutane rings **2a-2i**. The appearance of several peaks (up to 5) means that each product is obtained as the mixture of several isomers. The distribution of isomers and the relative amounts of each one in the mixture are collected in Table 1. In almost all cases the starting product is fully converted in the mixture of cyclobutanes. Therefore, quantitative yields of the cyclobutanes **2b**, **2c**, **2d**, **2e**, **2f**, **2g** and **2i** were obtained after simple evaporation of the reaction solvent. The only exceptions we have found are the unsubstituted oxazolone **1a** and the 4-nitro substituted oxazolone **1h**, with maximal conversions achieved of 49% and 75%, respectively. It seems that the presence of electron-donating substituents favor the reaction.

The characterization of the different isomers on each mixture has been carried out by a combination of spectroscopic (NMR) and X-ray diffraction methods. Figure S3 in SI shows the number of all possible isomers arising from [2+2] cycloaddition of *Z*- and *E*-oxazolones, their symmetry, and the chemical equivalence (or not) of key points of the molecules, such as the CH protons on the cyclobutane ring or the carbonyl groups. The analysis of the NMR spectra of cyclobutane **2b** is representative of the methodology followed for all cyclobutanes. The ^1H NMR spectrum shows five different peaks in the 4.5-5 ppm region (S38-S39, SI), corresponding to the CH protons in positions 2 and 4 of the cyclobutane ring of four different isomers, as discussed below. The values of the respective integrals give a composition (%) of the mixture

2b1/2b2/2b3/2b4 = 68.0/18.0/9.8/4.2. The ^{13}C NMR spectrum of the mixture shows the disappearance of the olefinic carbons of the $\text{CH}=\text{C}$ moiety in **1b** and the presence of two sets of new peaks in the Csp^3 zone, one set about 55-60 ppm and the other set in the 75 ppm region, assigned to the CH and the quaternary C atoms of the cyclobutane, respectively. These data confirm the formation of the cyclobutane ring.

Crystallization in $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ of this mixture allowed to separate a first crop of crystals, which contained exclusively the major isomers **2b1** and **2b2** (S40-S43, SI). The ocular inspection of this mixture showed to be composed by well-shaped colorless cubes and small colorless needles, which were easily separated manually. The NMR of the cubes corresponded to the pure isomer **2b1**, while the NMR of the needles matched with that of the second more abundant isomer **2b2**. However, no additional structural information about **2b1** and **2b2** could be gathered from their ^1H and ^{13}C NMR spectra due to the high symmetry reflected by the NMR, which could be explained by several possible isomers. In fact, following Figure S3, all isomers arising from coupling of two *Z*-oxazolones match with these NMR data [1,3-*ZZ*-syn (epsilon), 1,3-*ZZ*-anti (alpha), 1,2-*ZZ*-syn (beta), and 1,2-*ZZ*-anti (delta)], as well as all those coming from the coupling of two *E*-oxazolones [1,3-*EE*-syn (peri), 1,3-*EE*-anti (alpha), 1,2-*EE*-syn (omega), and 1,2-*EE*-anti (mu)]. Therefore, the determination of the structure of the two major isomers **2b1** and **2b2** was definitely carried out by X-ray diffraction methods, which will be discussed later below.

On the other hand, after repeated fractional crystallizations of **2b1** and **2b2** in $\text{CH}_2\text{Cl}_2/n$ -pentane, we obtained a solution more concentrated in the minor isomers **2b3** and **2b4** (SI S44-S46). In these cases, the NMR spectra provide more insights about the molecular structure. The analysis of the ^1H and ^{13}C NMR spectra of this mixture reveals clearly that the third isomer (**2b3**) has two equivalent CH protons and two non-equivalent Cq atoms, as it is evident from the ^1H - ^{13}C HMBC

correlation spectrum (S46, SI). There, the peak at 4.69 ppm in the ^1H NMR spectrum (CH) correlates with two different quaternary Cq atoms (78.52 and 73.12 ppm) in the ^{13}C NMR spectrum, and the same fact can be observed in the carbonyl region (178.37 and 172.57 ppm). This means that isomer **2b3** has symmetric (equivalent) CHAryl fragments, while the oxazolone rings are non-equivalent. As it is evident from Figure S3, only the epi-isomer,^{26,27} formed by a head-to-tail *syn*-cycloaddition of one (*Z*)-oxazolone with one (*E*)-oxazolone (see also Figure 4), accounts for the symmetry requested by the NMR spectra. It is remarkable that a (*Z*)-to-(*E*) isomerization step previous to the photocycloaddition is mandatory in order to obtain the epi-isomer **2b3**. This means that, under the reaction conditions, both [2+2]-cycloaddition and (*Z*)-to-(*E*) isomerization processes are taking place. The small amounts of **2b3** (and **2b4**) detected in the reactions suggest that cycloaddition is the main process and that isomerization is marginal. This will be evident from the molecular calculation of the (*Z*)-to-(*E*) isomerization step by DFT methods (see below). In the same line of analysis, the NMR data of the fourth isomer **2b4** shows two non-equivalent CH protons (4.79 and 4.94 ppm) in the ^1H NMR spectrum, both correlating with two equivalent Cq atoms (73.48 ppm) as well as with two equivalent carbonyls (175.74 ppm) in the ^{13}C NMR spectrum, as it is evident in the ^1H - ^{13}C HMBC correlation spectrum. In this case, we have equivalent oxazolones but non-equivalent C(H)Aryl moieties. Following Figure S3, only the gamma-isomer (see also Figure 4),^{26,27} formed through a head-to-tail *anti*-cycloaddition of one (*Z*)-oxazolone and one (*E*)-oxazolone, has the adequate symmetry to account for the observed spectra.

On the light of the spectroscopic analysis made in **2b** for **2b3** and **2b4**, a similar analysis has been performed in compounds **2c-2i**, which were characterized using analogous arguments through ^1H - ^{13}C HMBC heterocorrelations. In those cases where the amount of these minor third and fourth isomers was relevant (more than 10% altogether) the HMBC showed exactly the same

pattern of heterocorrelations (compounds **2c**, **2e**, **2f**, **2g**, and **2i**), showing that the structural assignation made for **2b3** and **2b4** is general and, therefore, that the third isomer in the studied complexes (**2c3**, **2e3**, **2f3**, **2g3**, **2i3**) correspond to the epi-isomer, and the less abundant fourth isomer (**2c4**, **2e4**, **2f4**, **2g4**, **2i4**) is the gamma-isomer.

In order to get more structural information, we crystallized several examples of the two major isomers (SI). In addition to compound **2b1** mentioned previously, we obtained crystals of the major isomers in the cases of **2e1** and **2f1**, and we were also able to grow crystals of the second most abundant isomer for compounds **2d2** and **2f2**. Once one crystal was selected from a crop of crystals for X-ray measurement, its identity was established by measuring the ^1H NMR spectrum of the remaining crystals and comparing their chemical shifts with those found in the original mixture. The X-ray structures of major isomers **2b1**, **2e1**, and **2f1** are presented in Figures 5, 6 and 7, respectively, while those of the second isomers **2d2** and **2f2** are shown in Figures 8 and 9.

Figure 5. Compound **2b1·OEt₂** (from **2b**: epsilon isomer as major isomer)

Figure 6. Compound **2e1** (from **2e**: epsilon isomer as major isomer)

Figure 7. Compound **2f1·2CH₂Cl₂** (from **2f**: epsilon isomer as major isomer)

As is evident from Figures 5, 6 and 7, the major isomers **2b1**, **2e1** and **2f1** are the epsilon (ϵ)-isomers, while from Figures 8 and 9 it is clear than isomers **2d2** and **2f2** have crystallized as alpha (α)-isomers (Table 1 and Figure 4), according with the original assignment of isomers by Stoermer and Bachér.^{26,27} The tricyclic structures in **2b1**, **2e1** and **2f1** show a very similar arrangement, originated by the head-to-tail *syn*-dimerization of the corresponding (*Z*)-oxazolones. As a result, the two carbonyl groups in positions 1 and 3 are in *cis* conformation, as are the two aryl rings in positions 2 and 4. However, the carbonyl and the aryl groups are mutually *trans*. As a whole, the configuration of these molecules is 1,2-*cis*-2,3-*cis*-3,4-*cis*. In this way, all phenyl rings in the molecules are pointing to the same side of the respective cyclobutane rings, and all of them are more or less perpendicular to it, giving to these molecules the appearance of a paddle wheel. The cyclobutane rings are not planar, showing a folded structure as defined by the values of the respective dihedral angles: C1-C10-C18-C27 and C10-C18-C27-C1 for **2b1** (20.5(2)° and -20.4(2)°) and C3-C4-C21-C22 and C4-C21-C22-C3 for **2f1** (22.8(2)° and -22.9(2)°). These values are similar to others found in structurally related situations.^{15,19,28,29}

Figure 8. Compound **2d2** (from **2d**: alpha isomer as second isomer)

Figure 9. Compound **2f2** (from **2f**: alpha isomer as second isomer)

However, the tricyclic structures found in **2d2** and **2f2** show clearly that they are alpha isomers, according with Stoermer and Bachér,^{26,27} which have been originated by the head-to-tail *anti*-cyclodimerization of two (*Z*)-oxazolones. In these structures the two carbonyl groups in positions 1 and 3 are in *trans*, and the same occurs with the two aryl rings in 2 and 4 positions. As a result, each carbonyl has one aryl in *cis* and the other one in *trans*, each aryl has one carbonyl in *cis* and another one in *trans*, and the configuration of both molecules is 1,2-*cis*-2,3-*trans*-3,4-*cis*. The cyclobutane rings in these compounds are planar, as it is usually observed for other cyclobutanes containing this arrangement of groups, as are, for instance, the alpha-isomers of the truxillic acid and their related derivatives.^{25,30-32} In all cases, the values of the internal parameters for bond distances (Å) and bond angles (°) are as expected, and they do not show unusual deviations from values found in the literature for related structural arrangements.^{15,19,25,28-33}

Although we have not characterized by X-ray crystallography all major isomers of all photo-dimers **2a-2i** here presented we can say, with a high degree of confidence, that it is very likely that the most abundant isomers **2a1-2i1** are the epsilon-isomers and that isomers **2a2-2i2** correspond to alpha-isomers. An additional argument to support this assignation is based on the trends observed for the chemical shifts of the CH protons of the cyclobutane ring: all ^1H NMR spectra show, for these protons, the same pattern of peaks, where the most shielded proton is the most abundant, and corresponds to the epsilon-stereoisomer, and the most deshielded proton is the second more abundant and corresponds to the alpha-isomer. On the other hand, the spectroscopic (NMR) characterization of minor isomers **2a3-2i3** and **2a4-2i4** (where the isomers are detectable) for the whole set of cyclobutanes allows us to conclude that **2a3-2i3** are the epi-isomers while **2a4-2i4** are the gamma-isomers.

Once the structural characterization was done, we have attempted a further optimization of the reaction, through a screening of solvents and wavelengths. The reactions have been monitored by ^1H NMR, therefore the optimization has been carried out in deuterated solvents. We have used Z-oxazolone **1b** as starting material and irradiated during 24 h. After this reaction time, none of the reactions reached completion. We have found that the reaction takes place selectively through a photocycloaddition pathway only when irradiated with blue light (465 nm), while photocycloaddition and extensive (Z)-(E) isomerization were observed when irradiated with ultraviolet light (405 nm), as previously reported.²²⁻²⁴ No reaction was observed when green (525 nm) or red (625 nm) lights were used.¹⁹ On the other hand, the results of the screening of different solvents, under the same reaction conditions (same concentration, 465 nm, 24 h), are resumed in Table 2. It is remarkable that the reaction takes place in all attempted solvents, except in methanol, regardless their more or less polar/apolar character.

Table 2. Screening of solvents with oxazolone **1b** to give cyclobutanes **2b1-2b4**.^a

Entry	Solvent	1b ^b	2b1	2b2	2b3	2b4
1	CD ₂ Cl ₂	9.8	61.5	15.7	9.0	4.0
2	CDCl ₃	12.7	54.7	18.2	9.4	4.9
3	C ₆ D ₆	22.5	54.4	8.1	10.3	4.7
4	CD ₃ CN	56.2	29.2	6.7	5.0	3.0
5	(CD ₃) ₂ CO	20.2	53.5	12.7	9.0	4.6
6 ^c	(CD ₃) ₂ SO	16.6	37.4	19.8	14.1	12.1
7	CD ₃ OD	100	-	-	-	-

a: % after 24h of reaction time. b: unreacted oxazolone **1b**. c: integration at OMe due to overlapping of the CH signals

As it can be seen, the best conversion is achieved in CD₂Cl₂, although a very good result is also obtained in CDCl₃, which provides a very clean transformation. In the other solvents, substantial amounts of the starting oxazolone **1b** remains unreacted and, therefore, they were not considered as suitable for this reaction. It is worthy of note that the pattern of NMR signals is more or less similar in all cases, showing only small variations in chemical shifts. This means that the tendency observed in the distribution of isomers remains the same in all attempted solvents (see SI), being the most abundant the epsilon-isomer (**2b1**) and the alpha-isomer (**2b2**), and then the epi- (**2b3**) and gamma-isomers (**2b4**), although individual values for each isomer can be different. This gives proof of a very versatile and robust process.

2.- In-line monitoring of continuous-flow [2+2]-photocycloaddition of 2-phenyl-4-(het)aryliden-5(4*H*)-oxazolones 1b-d. Continuous-flow (micro)reactors enable an enormous acceleration of the reaction rate in comparison with the batch methodology.^{19,34,35} Specially, the

use of microfluidic devices for photochemistry reactions shows significant advantages in comparison with the conventional photochemistry approach, with the high surface-to-volume ratio enabling irradiation of the entire reaction solution, a maximum penetration of light even for concentrated solutions, a favored reagents diffusion and heat- and mass- transfer, resulting in higher reaction yields and reduction of reaction times.^{34,35} The use of continuous flow and microreactors allowed us to observed quantitative yields for the [2+2]-photocycloaddition of the ortho-palladated derivatives of (Z)-4-arylidene-5(4*H*)-oxazolones within residence times as short as 5-20 minutes, reducing reaction times of days to minutes.¹⁹ Focused on reducing the long reaction times (72 h) for the synthesis of the previously mentioned cyclobutanes **2**, as well as aiming to get more information about the formation of the different isomers, a novel methodology for an on-flow [2+2]-photocycloaddition of oxazolones **1b-d** that implies the integration of NMR spectroscopy as analytical tool for the in-line monitorization of the reaction progress is presented here. The integration of analytics in-line with continuous-flow microreactors is a very interesting approach that combines the key advantages inherent to continuous flow, i.e. enhanced mixing and diffusion and extreme control over the reaction conditions, to the large amount of information about the reaction progress provided by a hyphenated analytical technique.³⁶⁻³⁸ An in-line method refers to a setup where the whole reaction mixture passes through the analytical instrument and is continuously analyzed, providing information of the reaction progress all over the reaction time.

Microfluidic NMR chips show great potential for mass-limited samples and hyphenation.^{39,40} In previous papers, we reported (a) the use of a microfluidic NMR chip hyphenated to a continuous-flow microliter microwave irradiation system for in-line monitoring and rapid optimization of reaction conditions;⁴¹ more recently, (b) a microfluidic NMR chip hyphenated to a continuous-flow microreactor platform (Labtrix-Start) to extract information within a single on-flow

experiment, monitoring the reaction progress before, at the onset of, and during the steady state of the reaction.⁴² Here, we report the development of a hyphenated system to monitor a photoreaction by means of small-volume NMR techniques (*micro-photo-NMR* setup), with the main purpose of extracting information about the formation of the different reaction isomers along the monitoring time. To the best of our knowledge, there is not a previous example in the literature for in-line monitoring of a photochemical process by NMR spectroscopy. UV-vis absorption spectroscopy has already found its application in the integration with a photochemistry reactor,³⁶⁻³⁸ but NMR spectroscopy is much more informative, e.g. providing qualitative and quantitative information.

For the development of the *micro-photo-NMR* setup, a glass microreactor (Labtrix Start, Chemtrix) (20 μ L active volume) containing the corresponding oxazolone was irradiated at 465 nm with a Printed Circuit Board (PCB) of LEDs (1W). LEDs are inexpensive, efficient and versatile energy sources for small dimension reactors. The use of microscale light sources as LEDs provides high photonic efficiency and high photon flux on the microchannels resulting in a very efficient reactor system.^{34,35} The tubing (outlet) of the microreactor was connected to a microfluidic NMR chip through microfluidic connections. The dimensions of the microfluidic NMR chip define a volume underneath the microcoil of 25 nL (detection volume). Figure 10 shows a schematic of the micro-photo-NMR setup.

Figure 10. Scheme of the *micro-photo-NMR* setup. A 20 μL microreactor hyphenated to a 25 nL active volume microfluidic NMR-chip. There are 10 μL dead volume between reaction and detection. The microreactor is irradiated by a PCB of LEDs with an optical output power of 1 W. The in-line monitorization of the cycloaddition progress results in a collection of NMR spectra within monitoring time, revealing the formation of up to four isomers.

Focused on extracting information along the reaction progress, we chose the active volume of the NMR chip much smaller with respect to that of the reactor volume, as previously reported^{41,42} to allow the analysis of the different zones of the reaction volume separately and continuously by the NMR chip, sampling multiple data points from a single on-flow experiment. Hence, the reactor volume and the flow rate were optimized for reaching a residence time that resulted in high reaction conversions. A reaction volume of 20 μL and a flow rate of 0.66 $\mu\text{L min}^{-1}$ for a solution of oxazolone **1b** defined a residence time of 30 minutes. The NMR acquisition parameters for a sufficient signal to noise ratio (SNR) even for low reaction conversions resulted in 3 minutes experiment time. Therefore, around 10 data points could be sampled from a single on-flow experiment. While on-flow, the LEDs were switched on, in-line, remote monitoring of the reaction

progress resulted in a collection of NMR spectra (Figure 11), which represent the disappearance of the reactant with time, and shows the appearance of the three forming isomers with different intensities (4.5, 4.6 and 4.8 ppm). The fourth isomer is not visible in this graph because of sensitivity issues since its conversion is very low, as revealed an analysis of a sample aliquot from this reaction by conventional 5 mm NMR tubes. Thus, every spectrum shown in Figure 11 corresponds to a subdivision of the reaction volume, recorded as the accumulated number of scans on-flow, in other words, to zones that have been irradiated with LEDs for different time. Regarding the monitorization of the different isomers, it is clear predominantly the high conversion shown by isomer 1 in comparison with isomers 2 and 3, the latter two isomers showing very similar in conversion. Remarkably, the high sensitivity achieved by the microfluidic NMR-chip enables the monitoring of a reaction product with a minimum amount of 5% of reaction conversion, what corresponds to 250 picomole of cycloaddition product in the 25 nL detection volume).

Figure 11. Left: Stacked spectra from the monitorization of the cycloaddition of oxazolone **1b** at 465 nm (* indicates product peaks while # refers to reagent peaks). All stacked spectra have been collected within a single on-flow experiment. Right: Reaction conversion for each isomer vs monitoring time for oxazolone **1b** at 465 nm. Considering a residence time of 30 minutes and an

NMR experiment time of 3 minutes, the number of data points defining the graphical of conversion versus reaction time is expected to be 10 as shown the total conversion graphical versus monitoring time before the steady-state of the reaction. ‡^{41,42}

Interestingly, the high surface-to-volume ratio of the microreactor (*micro-photo-NMR* setup) and the efficient illumination enhance the photocycloaddition of oxazolone **1b** resulting in quantitative reaction conversion for a residence time of only 30 minutes, in comparison to the 72 hours described in the first section.

Similar to oxazolone **1b**, the in-line monitoring of the photocycloaddition of oxazolone **1c** at 465 nm by means of the *micro-photo-NMR* setup reveals a collection of NMR spectra which shows the disappearance and appearance of the reagents and cycloaddition products, respectively, as a function of the monitoring time (Figure S1, left). Data extracted from the spectra are plotted as reaction conversion versus monitoring time (Figure S1, right), showing the isomer distribution from the first moments of the reaction. Once again, the high surface-to-volume ratio of microreactors together with an efficient illumination favors the reaction progress and enhances dramatically the reaction rate. Thus, quantitative yields are observed for a residence time of 30 minutes in the microreactor.

Similar results were observed for oxazolone **1d** after irradiation with a PCB of LEDs at 465 nm for 30 minutes by means of the *micro-photo-NMR* setup, although the reaction conversion was not complete as only 58% was observed. The in-line monitoring of the cycloaddition reaction progress for **1d** shows the formation of the four expected isomers within time (Figure S2). As observed before, the monitoring is possible even when very low amounts, i.e. hundreds of picomole, of

reaction product are formed (less than 5% reaction conversion is detectable in the active volume of 25 nL).

3.- Mechanism determination of the [2+2]-photocycloaddition using DFT methods. We then turned our attention to DFT calculations, in order to shed light into the reaction mechanism, taking special care to explain the reasons underlying the selectivity observed in the formation of the four head-to-tail cyclobutane rings (epsilon, alpha, epi and gamma). The substrate **1a**, bearing an unsubstituted phenyl ring was used as a computational model for its simplicity. The calculations were performed using the M06-2X⁴³ functional as implemented in Gaussian 09.⁴⁴ Optimizations were carried out in a solvent model (SMD, solvent = dichloromethane),⁴⁵ and the energies showed are Free Gibbs energies in kcal/mol as single points computed with def2tzvpp basis set on 6-31G(d) optimized structures (for more information, see SI material).

We confirmed initially that the *Z*-isomer of the oxazolone **1a** is more stable than its *E* analogue by as much as 3.9 kcal/mol. This value is significant since, as mentioned before, the appearance of epi and gamma adducts in the final mixture involves the participation of (*E*)-**1a** in the reaction. Thus, the light-induced isomerization of oxazolone competes with the cycloaddition in the experimental conditions. However, such a large stability difference (3.9 kcal/mol) corresponds to a marginal participation of (*E*)-**1a** in the equilibrium mixture of ca. 0.1%. The relative stabilities of the final adducts **2a** were also computed (Figure 12), showing a disagreement with the experimental product ratios. Although it is true that the most stable isomer *epsilon* **2a1** is also the major product of the reaction, the stability order of the rest of the isomers does not correspond with the experimental *epsilon* > *alpha* > *epi* > *gamma* experimental prevalence (Table 1).

Therefore, these values help discarding the equilibrium distribution of the final **2a(1-4)** isomers, and the reversibility of their formation processes.

Figure 12. Relative energies of the four head-to-tail adducts observed experimentally computed at M062X/def2tzvpp/CH₂Cl₂//M062X/6-31G(d)

We looked then into kinetic reasons to explain the selectivity of the reaction, and three reaction pathways were envisioned that could account for the double C-C bond formation: concerted and through diradicals, either in triplet or singlet state. Mechanistically speaking, the reaction can be stepwise (**TS1a**, figure 13) with formation of a diradical intermediate. In this case, both the transition state and subsequent intermediate can be in the singlet or triplet spin state. The [2+2] cycloaddition can also take place in a concerted manner through the **TSa**, shown in Figure 13. As outlined in figure 13 for the formation of the *epsilon* (ϵ) adduct from oxazolone (*Z*)-**1a**, the differences in the activation energies for the three processes are quite large, being the stepwise *singlet* process (**TS1a** ϵ -*Z,Z-s*, 48.7 kcal/mol) the lowest in energy and the only one that seems to be operative. The activation energy is perfectly achievable under light irradiation in the experimental conditions. The other two transition states (**TSa** ϵ -*Z,Z* and **TS1a** ϵ -*Z,Z-t*) are much higher in energy and can be safely discarded.

Thus, our mechanistic proposal is initiated with the formation of a singlet excited state of one oxazolone unit upon light irradiation, which reacts with a second alkene molecule by donation into its empty LUMO orbital through **TS1a** (Figure 14). A meta-stable singlet diradical intermediate is

formed (**INT1a** ϵ -**Z,Z-s**), which evolves almost barrierless through transition state **TS2a** to the final cyclobutane adduct. Although **TS2** is slightly higher in energy than **TS1**, the difference is meaningless and does not affect the above statements. The three structures (**TS1**, **INT1** and **TS2**) lie in an almost flat area of the potential energy surface, which can be considered altogether the rate limiting step.

Figure 13. Three reaction modes and their activation Free Gibbs energies for the *epsilon* adduct, computed at M062X/def2tzvpp/CH₂Cl₂//M062X/6-31G(d)

Figure 14. Energy profile for the formation of cyclobutane **2a** (ϵ) from (Z)-oxazolone **1a**

Finally, following this mechanistic proposal, we compared the energies of the processes leading to the four final adducts, in order to check if it can explain the observed reaction selectivity. Indeed, the lowest activation barrier (< 50 kcal/mol, Table 3) corresponds to the major *epsilon* (**2a** ϵ) isomer of the reaction, followed by the *alpha* and *epi* transition states, which show activation barriers around 53 kcal/mol. The slowest reaction would involve the *gamma* adduct (56.8 kcal/mol), which is in fact the minor isomer of the reaction, and even not detected in the case of **2a** (Table 1). These data are in qualitative agreement with the experimental selectivity observed in the cycloaddition.

Table 3. Absolute Free Gibbs energies in kcal/mol for the formation of the four isomeric final adducts through the stepwise mechanism.

Entry	TS1a	INT1a	TS2a	2a
epsilon	48.7	46.8	49.1	16.2
alpha	53.2	52.9	53.0	20.0
epi	51.5	49.9	53.2	17.7
gamma	52.6	50.7	56.8	20.7

CONCLUSIONS

The efficient synthesis of cyclobutane precursors of 1,3-diaminotruxillic acid derivatives has been achieved by direct [2+2]-photocycloaddition of (*Z*)-4-arylidene-5(4*H*)-oxazolones. The reaction takes place in CH₂Cl₂ solution, at room temperature, under irradiation with the blue light (465 nm) provided by low-power LED lamps (1 W) during 72 h. The cyclobutanes can be obtained in yields around 100% in most of the cases as a mixture of four isomers. The full characterization

of the mixtures shows that the most abundant isomer is the epsilon-truxillic, and that the alpha-, epi-, and gamma-isomers are also obtained but in lower amounts. This method represents the first efficient synthesis of truxillic derivatives by photodimerization, and paves the way for future photochemical research in this area.

The design of a setup for the coupling of a continuous-flow microreactor to a small-volume NMR probe has enabled the in-line monitoring of up to four cycloadduct isomers within time, and the optimization of reaction conditions showing a reduction of reaction times to only 30 minutes to yield quantitative conversions for certain oxazolone derivatives. This setup illustrates for the first time the feasibility of in-line monitoring of photo-activated reactions by NMR techniques.

DFT studies have been carried out and support a stepwise cycloaddition mechanism, with independent formation of the two new C-C bonds through a transient diradical singlet intermediate. At the same time, the concerted [2+2] cycloaddition or the involvement of triplet excited states can be safely ruled out. This mechanistic hypothesis is also able to account for the observed experimental selectivity in the formation of the final adducts, which are not in thermodynamic equilibrium.

ASSOCIATED CONTENT

Supporting Information. Full experimental section, supplementary figures S1, S2 and S3, copies of ^1H , ^{13}C NMR spectra and 2D correlations, xyz coordinates of all calculated species.

The following files are available free of charge: Supporting Info Truxillics (PDF)

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