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Synthesis of Triazole Derivatives of Levoglucosenone As Promising Anticancer Agents: Effective Exploration of the Chemical Space through *retro*-aza-Michael//aza-Michael Isomerizations

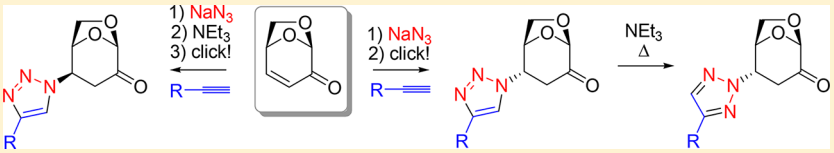
Yi-hsuan Tsai,[†] Carla M. Borini Etichetti,[‡] Carolina Di Benedetto,[‡] Javier E. Girardini,[‡] Felipe Terra Martins,[§] Rolando A. Spanevello,[†] Alejandra G. Suárez,[†] and Ariel M. Sarotti*[,†]

[†]Instituto de Química Rosario (IQUIR, CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario 2000, Argentina

[‡]Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Ocampo y Esmeralda, Rosario 2000, Argentina

[§]Instituto de Química, Universidade Federal de Goiás, Campus Samambaia, Goiânia, GO CP 131, 74001-970, Brazil

Supporting Information



ABSTRACT: The design and synthesis of biomass-derived triazoles and the *in vitro* evaluation as potential anticancer agents are described. The discovery of base-catalyzed *retro*-aza-Michael//aza-Michael isomerizations allowed the exploration of the chemical space by affording novel types of triazoles, difficult to obtain otherwise. Following this strategy, 2,4-disubstituted 1,2,3-triazoles could be efficiently obtained from the corresponding 1,4-disubstituted analogues.

INTRODUCTION

The need to achieve sustainable development for the future generations has been motorizing the scientific research in the last 25 years, both in the academia and industry. Due to the increased concern for environmental, economic, and geo-politics, the demand for cleaner fuels and chemicals has significantly impacted the chemical sector. The use of biomass as a renewable source of supply of organic compounds represents by far the most convenient and deeply studied alternative to untie our oil dependence.[1] In this regard, vegetal biomass is particularly suitable to accomplish these goals. Not only is it generated in impressive amounts (170 billion metric tons a year) but also allows CO_2-fixation and O_2-release during the photosynthesis process.[1b] Among the wide variety of organic compounds elaborated by plants, carbohydrates are the most abundant ones (75%), and for that reason, are the most prominent renewable feedstocks for the production of chemicals.[1]

Several strategies have been developed for the transformation of sugars into valuable bioproducts, including fermentation, dehydration, hydrolysis, esterification, oxidation, and pyrolysis processes, among others. The pyrolysis of biomass represents an area of fervent development currently, giving rise to several chemical platforms depending on the reaction conditions employed during the thermal decomposition stage.[2] The pyrolytic treatment of acid pretreated cellulose-containing materials yields levoglucosenone (1,6-anhydro-3,4-dideoxy-β-D-glycero-hex-3-enopyranos-2-ulose, **1**), a highly attractive chiral synthon (Figure 1).[3]

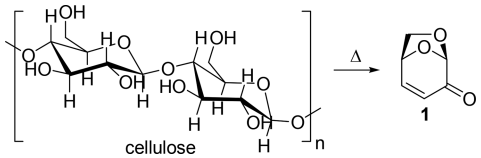


Figure 1. Pyrolytic transformation of cellulose into levoglucosenone (**1**).

Due to its rigid structure and versatile functionality, levoglucosenone has been extensively employed in the recent past as a starting material for the synthesis of natural products,[3a,c,4] valuable intermediate synthons,[3,5] and in the development of new tools of asymmetric synthesis (including chiral auxiliaries, ligands, and organocatalysts).[3b,c,6] Moreover, its hydrogenated derivative, commercially termed Cyrene, is a solvent with a high potential, on which extensive research is being conducted.[7]

The utility of levoglucosenone in the field of medicinal chemistry has also been explored.[3a,c,8] Perhaps the most important discoveries are related to the promising anticancer activities exhibited by many of its derivatives.[8] For instance, the

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group of Peri developed new Ras inhibitors from levoglucosenone-derived isoxazolidines, showing an interesting toxicity against several tumor cell lines.[8a] On the other hand, the group of Wiman proved that 4-substituted-dihydrolevoglucosenone derivatives (easily obtained from the direct Michael addition of heterocyclic nucleophiles to the highly reactive enone system present in **1**) also displayed a high antiproliferative activity when tested in several cell lines *in vitro*. Interestingly, they also found an increasing cytotoxicity in cancer cells expressing p53 point mutants (in comparison with the corresponding counterparts lacking p53 expression), providing convincing evidence in line with a pharmacological restoration of p53 activity.[8b] The suitability of 4-substituted derivatives of levoglucosenone as anticancer agents was also found by the Witczak group by decorating the C-4 position with different thio-sugars.[8d,e] We recently found that other 4-sulfurated derivatives of **1** also displayed a high cytotoxicity when tested against hepatocarcinoma cell lines, and in agreement with the Wiman group findings, we noted that Huh-7 cell lines (with mutated p53 gene) were more susceptible to the *in vitro* treatment than HepG2 cell lines (expressing endogenous wt p53).[8c]

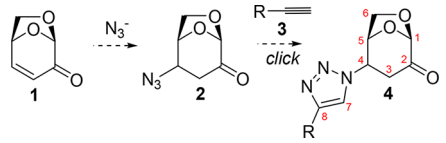
Briefly, p53 is a transcription factor that acts as a tumor suppressor. When DNA damage or oncogenic signals are detected, p53 triggers a complex response including cell cycle arrest and/or DNA repair, as well as partial reprogramming of cell physiology. Severe DNA damage or persistent oncogenic stress may induce irreversible processes such as programmed cell death (apoptosis) or senescence, in order to eliminate cells prone to malignant transformation.[9] In line with this role, mutation of the p53 gene (*TP53*) is the most frequent genetic alteration in human cancer, exceeding 50% of cases in some tumor types.[10] A hallmark of p53 alteration is the presence of missense mutations, which are found in more than 70% of cases, allowing abundant expression of point mutant proteins. Mutations abrogate DNA binding and tumor suppressor function. Interestingly, the presence of a full length p53 mutant protein in tumors suggested the possibility to restore the wt (wild-type) function through refolding induced by the interaction with small organic molecules. This approach represents a leading strategy in drug discovery that allowed the identification of PRIMA-1 as a pioneering compound, followed by other molecules, some of which have reached clinical trials.[9,11]

On the basis of this exciting background, it becomes clear that the search of new derivatives of levoglucosenone with anticancer properties is worthwhile both from the medicinal and sustainable chemistry aspects. The exploration of the chemical space through the generation of diversity represents a key step in the arduous path toward new drugs and has been significantly helped by modern click chemistry approaches.[12] In particular, the synthesis of 1,2,3-triazoles has emerged as one of the leading strategies to easily introduce structural diversity in organic molecules.[12b] There are several features that make the triazolic core pharmacologically important, including chemical stability, aromaticity, high dipolar moment, and the ability to participate actively in hydrogen bond formation and other interactions as well (such as dipole−dipole and π-stacking) that, in turn, facilitate the binding with the biological targets and improve the solubility.[13] Moreover, they can be easily obtained through the 1,3-dipolar cycloaddition reactions between alkynes and azides under Cu(I) catalysis, among the most emblematic reactions within the click chemistry paradigm.[12b] Not surprisingly, the number of bioactive compounds bearing a 1,2,3-triazole motif has significantly increased in the last decades, covering a wide variety of biological activities,[13] including anticancer ones.[13,14] Hence, we have been encouraged to design a simple and efficient strategy for the synthesis of chiral triazole compounds derived from levoglucosenone and evaluate their cytotoxicity against human breast cancer cells bearing a missense mutation in p53.

RESULT AND DISCUSSION

Synthesis of C-4-α-1,4-Disubstituted-1,2,3-triazolyl Derivatives. By taking advantage of the well-known high reactivity of the α,β-unsaturated system present in **1** as a Michael acceptor,[3] we foresaw that the installation of an azide group at C-4 generates **2**, which upon treatment with different alkynes **3** under Cu(I) catalysis would afford the desired 1,2,3-triazole derivatives **4** (Scheme 1).

Scheme 1. Proposed Strategy for the Synthesis of the Desired Triazoles 4



Among the different protocols for the β-azidation of enones, the most common ones rely on the use of 3−5 equiv of NaN_3 or $TMSN_3$ as an azide source, 3−5 equiv of an acid (typically AcOH or HCl) to smoothly generate *in situ* the reactive HN_3 species, and a base (NEt_3, DABCO, etc.) to catalyze the conjugate addition in a suitable solvent (CH_2Cl_2, H_2O, ionic liquids, etc.), demanding between 5 and 20 h at room temperature to afford the desired product in high yields.[15] However, using such experimental procedures in our case yielded the desired azide **2** in a modest conversion (up to ∼60%) as determined by ^1H NMR analysis of the reaction crude material. We also tested the experimental conditions developed by Horton et al. for the β-aziridation of isolevoglucosenone (NaN_3, TFA, THF),[15f] but the conversion slightly improved (73%). After several trials, we were able to enhance the conversion up to 81% upon increasing the amount of AcOH (4 equiv of NaN_3, 40 equiv of AcOH, 0.2 equiv of NEt_3, CH_2Cl_2, 12 h). This outcome led us to evaluate AcOH directly as a solvent, and to our delight, excellent levels of conversion (∼100%) were achieved in only 10 min of reaction time (3.4 equiv of NaN_3, 0.14 equiv of NEt_3, AcOH, 10 min). However, all efforts to purify **2** by column chromatography were met with no success, as significant *retro*-aza-Michael process took place, leading to large quantities of **1** and low amounts of **2** contaminated by other decomposition by-products. Nevertheless, we could manage to isolate a sample of reasonable purity for NMR characterization, observing all of the signals expected for **2**, including two additional sp^3-hybridized carbons at δ_C 36.5 ppm (CH_2, C-3) and 59.7 ppm (CH, C-4). The stereochemistry at C-4 was determined from the coupling constants between H-3_{ax}/H-4 (6.6 Hz) and H-3_{eq}/H-4 (∼0 Hz), indicating axial−equatorial and diequatorial relationships, respectively, and was confirmed by NOE correlation between H-4 and H-6_{endo} (Figure 2). It is noteworthy that the aza-Michael addition proceeded with excellent levels of π-facial selectivity, suggesting that the steric hindrance exerted by the

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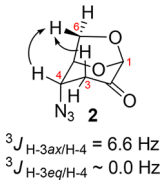


Figure 2. Structure of azide **2** with key NOE correlations.

1,6-anhidro bridge directed the exclusive attack of the nucleophile from the α-face of the molecule.[3]

Once the synthesis of **2** was optimized, we next explored the 1,3-dipolar cycloaddition with terminal alkynes to afford the corresponding triazoles **4**. Given the impossibility to purify **2**, the click reaction was evaluated with the crude mixture of **2** (which showed no trace of isomerized products *vide infra*, according to the ^{1}H NMR analysis of the reaction mixture) using a modification of the protocol developed by Kim and co-workers (1.3 equiv of alkyne, 14 mol % $CuSO_4{\cdot}5H_2O$, 40 mol % sodium ascorbate, 1:1 mixture of CH_2Cl_2/H_2O, 1 h).[16] By using phenylacetylene (**3a**) as a model alkyne counterpart, the desired triazole **4a** was obtained in a good overall yield (86%, 2 steps). The formation of the 1,4-disubstituted-1,2,3-triazole moiety was confirmed in the ^{13}C NMR spectra with two signals at δ_C = 148.4 ppm (C-8) and 118.1 ppm (C-7), characteristic for this type of aromatic nuclei.[17] The stereochemistry at C-4 was proposed from the coupling constants between H-4 with H-3$_{ax}$ (8.1 Hz) and H-3$_{eq}$ (~0 Hz) and was confirmed by NOE correlations between H-4 and H-6$_{endo}$. The structure of **4a** was finally established by X-ray diffraction analysis (Figure 3).

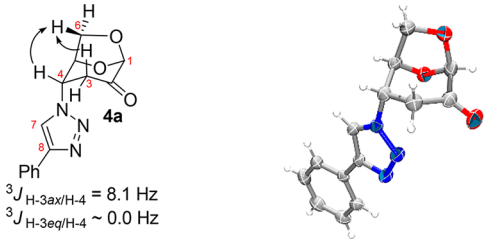
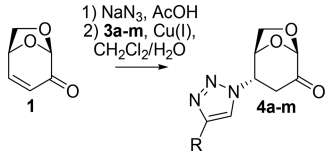


Figure 3. Left: structure of triazole **4a** with key ^3J couplings and NOE correlations. Right: ORTEP diagram of **4a** showing the displacement ellipsoids for the non-H atoms at the 30% probability level.

Next, we evaluated these reaction conditions with other terminal alkynes bearing diversity of alkyl, vinyl, and aryl substituents with different substitution patterns and heteroatoms. As shown in Table 1, good to very good overall yields were obtained in all cases under study. All of the newly synthesized compounds **4b–m** were characterized by standard spectroscopic studies, including 1D and 2D NMR experiments and showed close similarity to those described for **4a**. Interestingly, these reaction conditions afforded compounds **4a–m** as the only isolated triazole derivatives, showing no trace of isomerized products (*vide infra*) in the ^{1}H NMR analysis of the reaction mixtures.

Synthesis of C-4-β-1,4-Disubstituted-1,2,3-triazolyl Derivatives. During our preliminary optimization of the reaction between **2** and **3a**, we noticed that, in some cases apart from the desired triazole **4a**, variable amounts of a minor isomer **5a** was also formed. The signals at δ_C = 148.1 ppm (C-8) and 119.0 ppm (C-7) indicated the presence of a 1,4-disubstituted-1,2,3-triazole unit.[17] Moreover, most of the

Table 1. Synthesis of Triazoles 4a–m



entry	R	alkyne	yield (%, 2 steps)[a,b]
1	–Ph	**3a**	86
2	–CO_2Me	**3b**	76
3	–4-OMe–Ph	**3c**	83
4	–C_8H_{17}	**3d**	77
5	–CH_2OAc	**3e**	64
6	–CH=CHCH_2OAc	**3f**	65
7	–C(OH)Ph	**3g**	71[c]
8	–CH_2OPh	**3h**	65
9	–CH_2SPh	**3i**	87
10	–CH_2NHPh	**3j**	51
11	–CH_2–O-4-OMe–Ph	**3k**	82
12	–CH_2–O-2-NO_2–Ph	**3l**	84
13	–CH_2–S-4-Me–Ph	**3m**	70

[a]Step 1: NaN_3 (3.4 equiv), NEt_3 (0.14 equiv), AcOH. Step 2: **3a–m** (1.3 equiv), $CuSO_4{\cdot}5H_2O$ (14 mol %), sodium ascorbate (40 mol %), CH_2Cl_2/H_2O. [b]Yields correspond to isolated compounds after column chromatography. [c]Obtained as a ~1:1 inseparable mixture of the two epimers at C-9.

remaining NMR data showed similarity to those observed for **4a**, suggesting that both compounds were diastereoisomers. Analysis of the coupling constants between H-3$_{ax}$/H-4 (11.9 Hz) and H-3$_{eq}$/H-4 (6.5 Hz) indicated that **5a** was the epimer of **4a** at C-4. This observation was consistent with the significant deshielding observed for H-6$_{endo}$, and H-3$_{ax}$ ($\Delta\delta_{5a-4a}$ = 0.44 and 0.32 ppm, respectively), accounting for the anisotropy exerted by the aromatic groups directed toward the β face of the molecule. However, the lack of useful NOE correlations between H-6$_{endo}$ with the triazole or aromatic protons precluded the confirmation of our assignment. We next performed quantum calculations of NMR,[18] an approach that has been extensively employed in the recent past to settle structural issues of complex organic molecules.[19] The chemical shifts of **4a** and **5a** were computed at the PCM/mPW1PW91/6-31+G**//B3LYP/6-31G* level of theory (using chloroform as a solvent), and a very good agreement with the experimental data collected for those compounds was observed in each case, respectively. In particular, our calculations correctly reproduced the downfield shifts for H-6$_{endo}$ and H-3$_{ax}$ experimentally observed in the case of **5a** (calcd $\Delta\delta_{5a-4a}$ = 0.26 and 0.51 ppm, respectively). The computed DP4+ probability provided a high confidence in our structural proposal (see Tables S8 and S10 in the Supporting Information),[20] which was further confirmed by X-ray analysis of the hydrate form of **5a** obtained from a DMSO/EtOH mixture (2:1 v/v) of solvents (Figure 4).

With the structure of **5a** finally unraveled, further studies were conducted to understand the origins of its formation. After several trials, using the same reaction conditions for the click stage, we noticed that levoglucosenone was always present (as determined by TLC) in the crude azide that lead to the formation of **4a** and **5a** mixtures, while azides with unnoticeable amounts of **1** afforded **4a** as the only reaction product. These results suggested that the generation of **5a** depended somehow on the presence of **1**, which in turn would arise from a *retro*-aza-Michael decomposition of **2**. To verify our hypothesis, we

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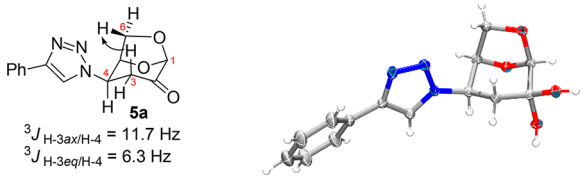
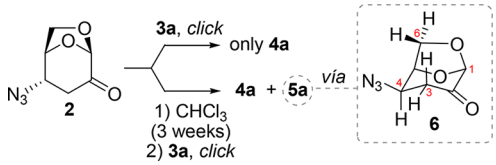


Figure 4. Left: structure of triazole **5a** with key ^3J couplings and NOE correlations. Right: ORTEP diagram of the hydrate form of **5a** showing the displacement ellipsoids for the non-H atoms at the 30% probability level.

performed two parallel experiments starting from the same crude mixture of freshly prepared azide **2** (Scheme 2). One

Scheme 2. Experiment Designed to Explain the Generation of 5a



sample was immediately reacted with **3a** under standard reaction conditions, and the other sample was left dissolved in chloroform for 3 weeks prior the click event. In the last case, we noticed significant amounts of **1** after TLC analysis, indicating that a *retro*-aza-Michael path took place. As expected, only the first sample afforded exclusively triazole **4a**, whereas the other sample yielded a mixture of **4a** and **5a**. According to the experimental evidence, we hypothesized that **5a** could be formed through an isomerization of **4a** or directly from azide **6**, generated by C-4 epimerization of **2**. The first option was ruled out as all attempts to transform **4a** into **5a** were met with no success. For instance, a pure sample of **4a** was recovered unchanged after being left dissolved for 2 weeks both at room temperature and 70 °C. Similar results were observed after submitting **4a** to the reaction conditions employed in the click stage.

Hence, we next set out to study the possible isomerization of **2** using NMR spectroscopy. First, we recorded the ^{1}H NMR spectra of **2** after allowing the sample to suffer *retro*-aza-Michael decomposition by gently stirring a $CDCl_3$ solution for 3 weeks. Interestingly, apart from the expected resonances of **1** and **2**, we noticed signals of a third compound that according to their chemical shifts and multiplicities were consistent to those expected for **6** (Table 2). The stereochemistry at C-4 was determined from the coupling constants between H-3$_{ax}$/H-4 (10.5 Hz) and H-3$_{eq}$/H-4 (7.4 Hz), indicating that the azide group was directed toward the β-face of the molecule. In addition, the ^{13}C NMR spectra of the mixture also reflected the appearance of a new set of signals that showed a close similarity to those collected for **2**. Given the impossibility to isolate pure samples of **6** due to decomposition in the chromatographic process, we computed the NMR shifts of **2** and **6** at the PCM/mPW1PW91/6-31+G**//B3LYP/6-31G* level of theory using chloroform as a solvent. As shown in Table 2, a very good match between experimental and computational data was observed for each pair. Our assignment was further supported by the DP4+ probability calculations, indicating that the structures proposed for **2** and **6** are the most likely ones in high

Table 2. Experimental ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Shifts of 2 and 6 Collected in $CDCl_3$ and Calculated Values at the PCM/mPW1PW91/6-31+G//B3LYP/6-31G* Level of Theory Using Chloroform As a Solvent**

	δ_{exp}		δ_{calcd}	
atom	**2**	**6**	**2**	**6**
H-1	5.19	5.10	5.13	5.02
H-3$_{ax}$	2.94	2.55	2.97	2.58
H-3$_{eq}$	2.59	2.81	2.50	2.68
H-4	3.91	4.23	3.81	4.12
H-5	4.76	4.64	4.72	4.61
H-6$_{endo}$	3.99	4.24	4.13	4.40
H-6$_{exo}$	4.03	3.89	4.16	4.04
C-1	101.3	99.8	100.4	99.1
C-2	196.2	ND	196.6	196.5
C-3	36.5	37.6	36.6	37.6
C-4	59.7	58.1	62.2	60.8
C-5	75.7	74.0	75.6	73.8
C-6	65.8	63.9	63.7	61.8

confidence (>99.9%, Tables S4 and S6 in the Supporting Information).

This finding provided a paramount opportunity to explore the chemical space of these types of compounds via the generation of C-4-β-triazolyl derivatives, which are difficult to synthesize otherwise. Therefore, we decided to perform the isomerization of **2** in a more reproducible and rapid fashion. It is well-known that the *retro*-aza-Michael reaction can be catalyzed by bases, and for that reason, we studied the transformation of **2** into **6** after the addition of catalytic amounts of NEt_3. The progress of the reaction was monitored by taking the ^{1}H NMR spectra at regular time intervals. In all cases, the different chemical shifts in the signals exhibited by **1**, **2**, and **6** allowed the determination of the progress of the reaction by integration of those signals in each ^{1}H NMR spectrum. As shown in Figure 5, at the early stages of the reaction, the *retro*-aza-Michael event took place predominantly, increasing the amount of **1** from 0 to 20% in less than 10 min. Next, the concentration of **1** remained almost constant, and the molar fraction of **2** rapidly diminished with the concomitant

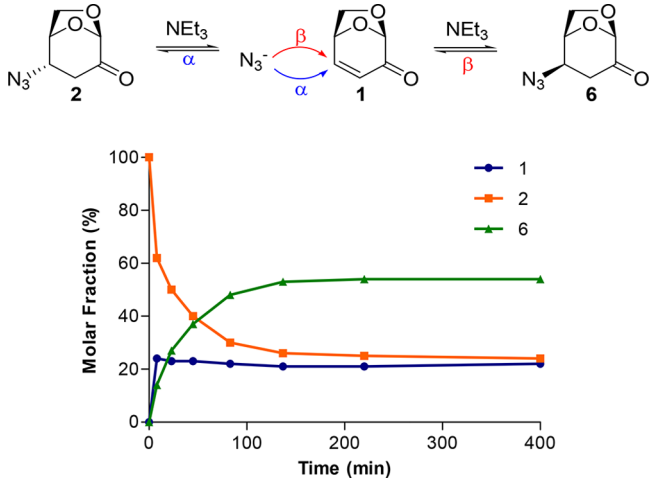


Figure 5. Concentration vs time plot observed for the epimerization of **2** to **6** using ^{1}H NMR spectroscopy. $[2]_0$ = 0.35 M, 15 mol % NEt_3, $CDCl_3$, 25 °C.

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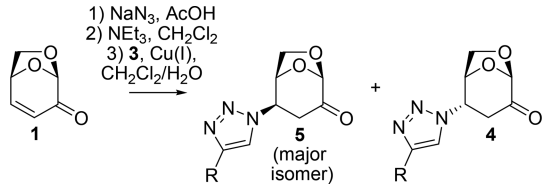
increase in the molar fraction of **6** until the equilibrium was reached at ~2 h. The final **6**/**2** ratio was 70:30, which was consistent with the higher stability computed for **6** at the PCM/B3LYP/6-311+G** level of theory (ΔE = 1.1 kcal/mol). In order to provide further validation to our mechanistic proposal, the relative energies of the competing transition structures (**TS-2** and **TS-6**) leading to azides **2** and **6**, respectively, were also computed at the M06-2X/6-31G* level. As shown in the Supporting Information, **TS-2** was 2.0 kcal/mol lower in energy than **TS-6**, whereas **2** was 1.1 kcal/mol higher in energy than **6**. In excellent agreement with our experimental findings, our DFT calculations clearly suggested that azides **2** and **6** should be the kinetic and thermodynamic products, respectively, of the *aza*-Michael aziridation of levoglucosenone.

With these results in hand, we next developed a new experimental protocol for the preferential generation of β-substituted triazoles **5**. Hence, the crude azide **2** (obtained following our optimized experimental procedure) was dissolved in CH_2Cl_2, and 10 mol % of NEt_3 was added. After stirring for 3 h at room temperature (to ensure the equilibration of the system), water was added and the 1,3-dipolar cycloaddition with different alkynes was carried out using the above-mentioned procedure. As shown in Table 3, in all cases, the

Table 3. Synthesis of Triazoles 5[a]



entry	R	alkyne	yield (%, 3 steps)[b]	ratio **5**/**4**
1	−Ph	**3a**	88	65:35
2	$-CO_2Me$	**3b**	82	71:29
3	−4-OMe-Ph	**3c**	80	75:25
4	$-C_8H_{17}$	**3d**	80	71:29
5	−C(OH)Ph	**3g**	70	65:35

[a]Step 1: NaN_3 (3.4 equiv), NEt_3 (0.14 equiv), AcOH. Step 2: NEt_3 (10 mol %), CH_2Cl_2. Step 3: **3a**−**m** (1.3 equiv), $CuSO_4{\cdot}5H_2O$ (14 mol %), sodium ascorbate (40 mol %), CH_2Cl_2/H_2O. [b]Yields correspond to isolated compounds after column chromatography.

desired compounds **5a**−**d**,**g** were obtained in very good overall yields, and as expected, the **5**/**4** ratios (~7:3) were in good agreement with the **2**/**6** ratio at the equilibrium determined by ^{1}H NMR. It is also noteworthy that each **5**/**4** pair could be easily separated by column chromatography, facilitating the purification for further bioassays.

Synthesis of C-4-α-2,4-Disubstituted-1,2,3-triazolyl Derivatives. While trying to elucidate whether **5a** could be formed by a C-4 epimerization of **4a** following a similar approach than that proposed for the transformation of **2** into **6**, we treated a pure sample of **4a** with 1 equiv of NEt_3 in $CHCl_3$ for 7 days at room temperature. Surprisingly, we did not observe any trace of **5a**, and **4a** was not recovered. Instead, we isolated a novel compound **7a**, whose ^{1}H NMR spectra was similar to **4a**. The coupling constants between H-3$_{ax}$/H-4 (7.3 Hz) and H-3$_{eq}$/H-4 (~0 Hz) indicated a C-4-α-substituted levoglucosenone derivative, confirmed by NOE interaction between H-4 and H-6$_{endo}$ (Figure 7). The ^{13}C NMR data was also similar to that of **4a**, except for the signal attributed to the triazole CH carbon (C-7) that appeared considerably deshielded in **7a** (131.4 ppm vs 118.1 ppm). On the basis of this finding, we first considered that **7a** might be the 1,5-disubstituted analogue of **4a** (compound **8a**, Supporting Information).[17] However, the quantum chemical calculations of the NMR shifts of such structure did not match well the experimental values. In particular, despite the fact that the C-7 resonance was correctly reproduced by our PCM/mPW1PW91/6-31+G**//B3LYP/6-31G* calculations ($\delta_{C\text{-}7}$ = 132.5 ppm), the C-8 signal ($\delta_{C\text{-}7}$ = 138.2 ppm) was placed much more shielded than that observed for **7a** ($\delta_{C\text{-}8}$ = 148.2 ppm). Intrigued by this result, we next searched in the literature for 1,2,3-triazoles bearing a different substitution pattern (compounds **9a**−**c**, Figure 6)[21] and found that, in line with

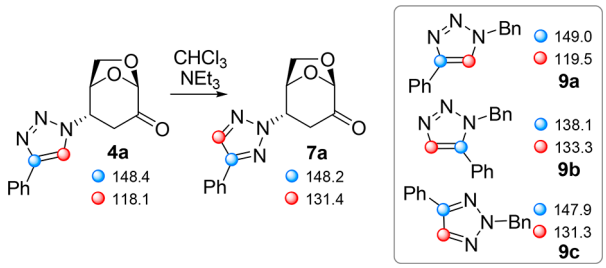


Figure 6. Basic promoted isomerization of **4a** into **7a** and relevant ^{13}C NMR chemical shifts of different triazole analogues.

our calculations, the quaternary carbon resonance of the 1,5-disubstituted triazole **9b** is 138.1 ppm, which is considerably different than the value observed for **7a**. On the other hand, the ^{13}C NMR data collected for the triazole carbons of compound **9c** (featuring a 2,4-disubstitution pattern) nicely matched the experimental shifts observed for **7a**. This finding was further supported after DFT calculations of the NMR shifts for the 2,4-disubstituted-1,2,3-triazole derivative **7a** (DP4+ > 99.9%, Table S12 in the Supporting Information).

Since **7a** is an oily compound prone to form glasses, in order to support our assignment by X-ray analysis, we considered that the reduction of the carbonyl group at C-2 might afford a crystalline compound. In fact, one of the two alcohols obtained after treatment of **7a** with $NaBH_4$ in MeOH could finally be crystallized after exhaustive trials of solvents and conditions, and its structure was unambiguously determined by X-ray diffraction analysis. As depicted in Figure 7, the 2,4-disubstituted-1,2,3-triazole unit is directed toward the α-face of the molecule, as suggested from experimental and theoretical NMR studies discussed above.

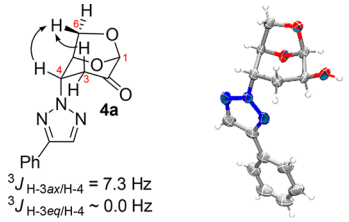


Figure 7. Left: structure of triazole **7a** with key ^3J couplings and NOE correlations. Right: ORTEP diagram of the β-alcohol derivative of **7a** showing the displacement ellipsoids for the non-H atoms at the 30% probability level.

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This serendipitous base-catalyzed isomerization of **4a** to **7a** not only provided a useful alternative to explore more deeply the chemical space in our preliminary screening of levoglucosenone derivatives as anticancer compounds but also represented a novel synthetic strategy for the preparation of N2-substituted 1,2,3-triazoles, for which extensive research has been conducted in the recent past.[22] In this regard, it is important to note that the isomerization of N1-substituted 1,2,3-triazolyl-ketones to the corresponding N2-substituted counterparts has been covered by Sharpless and co-workers,[22e] whereas the isolation of N2-substituted triazoles derived from metallic azides and alkynes has also been studied.[22f−h] However, the lack of reports on such isomerization when dealing with disubstituted metal-free triazole moieties motivated us to perform a comprehensive study on this system. Considering that the isomerization of **4a** was too slow at room temperature, we decided to explore the reaction at higher temperatures. After preliminary trials, we found that gentle heating at 70 °C afforded the isomerized product **7a** in more suitable reaction times (12 h), whereas higher temperatures led to some decomposition of byproducts. Different solvents and additives were next evaluated, and the results are collected in Table 4. In all cases, the solvent was evaporated and the crude

Table 4. Optimization for the Isomerization of 4a into 7a[a]

entry	solvent	additive (equiv)	ratio **1**/**4a**/**7a**[b]
1	MeOH	NEt_3 (0.2)	–
2	AcOEt	NEt_3 (0.2)	29:41:30
3	THF	NEt_3 (0.2)	30:36:34
4	hexane	NEt_3 (0.2)	0:73:27
5	$CHCl_3$	NEt_3 (0.2)	6:33:61
6	$CHCl_3$	NEt_3 (0.2)	27:39:34
7	$CHCl_3$	–	0:100:0
8	$CHCl_3$	AcOH (0.2)	4:96:0
9	$CHCl_3$	DBU (0.2)	–
10	$CHCl_3$	DIPEA (0.2)	24:40:36
11	$CHCl_3$	NEt_3 (0.5)	3:10:87
12	$CHCl_3$	NEt_3 (1.0)	3:3:94

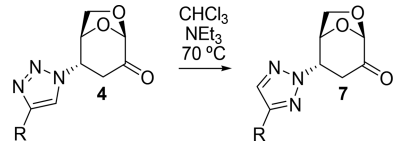
[a]All reactions were carried out at 70 °C for 12 h in Hach tubes. [b]Determined by integration of the ^{1}H NMR spectra of the crude mixtures.

mixtures were immediately analyzed by ^{1}H NMR to determine their composition. Interestingly, in all cases, we noticed variable amounts of **1**, suggesting that the mechanism of the isomerization involved a *retro*-aza-Michael path (*vide infra*).

Among the studied solvents, chloroform afforded the highest conversion toward the desired compound **7a** (entries 1−5). Moreover, we found that a base was crucial for the success of the reaction, affording unreacted **4a** when no additive was added (entry 7). Similar results were observed when using acetic acid as a catalyst (entry 8). We also tested other bases (entries 9 and 10), but unsatisfactory results were obtained. Finally, we explored the amount of NEt_3 (entries 5, 11, and 12) and found that the addition of 1.0 equiv of base afforded the optimal results. With this optimized procedure in hand, we next evaluated the isomerization reaction with 5 representative 1,4-disubstituted triazoles bearing different types of substituents in the triazole moiety. As shown in Table 5, the corresponding 2,4-disubstituted triazoles were obtained in good overall yields.

To have a better understanding of this isomerization process, we monitored the progress of the reaction by taking ^{1}H NMR

Table 5. Synthesis of Triazoles 7



entry	R	product	yield (%)[a]
1	−Ph	**7a**	86
2	$-CO_2Me$	**7b**	87
3	$-C_8H_{17}$	**7d**	71
4	−C(OH)Ph	**7g**	83
5	$-CH_2-O$-4-OMe−Ph	**7k**	71

[a]Yields correspond to isolated compounds after column chromatography.

spectra of **4a** at regular time intervals after the addition of 1 equiv of NEt_3 in $CDCl_3$ at 70 °C. As shown in Figure 8, during

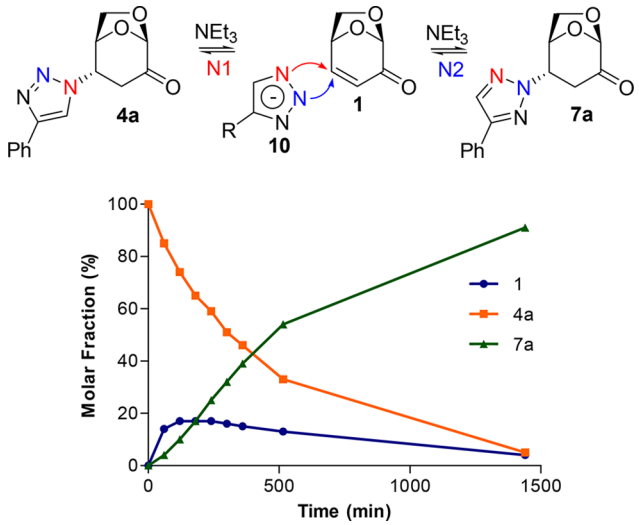


Figure 8. Concentration vs time plot observed for the isomerization of **4a** to **7a** using ^{1}H NMR spectroscopy. $[\mathbf{4a}]_0$ = 0.2 M, 1.1 equiv of NEt_3, $CDCl_3$, 25 °C.

the first 2 h, the only process that took place was the *retro*-aza-Michael decomposition of **4a** into **1**. Once levoglucosenone reached its maximum concentration (ca. 20%, similar to those observed for the isomerization of **2**), the molar fraction of **7a** started to increase its final value (91%).

With these data in hand, and in line with previous observations for related systems, we proposed that the conversion of **4** to **7** can be rationalized by assuming a reversible 1,4-conjugate addition under basic media (similar to those suggested by the isomerization of **2**). Hence, **4** should decompose via a *retro*-aza-Michael path to afford **1** and the corresponding triazole ion **10**, which would further attack the C-4 position of **1** through the N2 nitrogen atom to afford **7**. Our observations reveal that this compound should be the thermodynamically more stable product. In order to understand the kinetic/thermodynamic effects of this isomerization, the competing transition structures (**TS-4a** vs **TS-7a**) leading to products **4a** and **7a**, respectively, were computed at the M06-2X/6-31G* level of theory. In perfect agreement with our experimental findings, compound **7a** was 4.2 kcal/mol more stable than **4a**, whereas the energy trend was reversed for the corresponding transition structures (**TS-4a** 1.3 kcal/mol more

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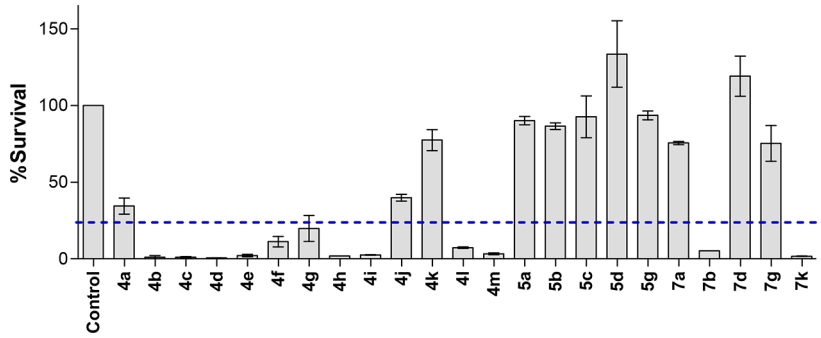

Figure 9. Effect of the levoglucosenone-derived 1,2,3,-triazoles library on survival of MDA-MB-231 breast cancer cells. Survival was determined using MTT bioreduction, normalized to control treatment (DMSO), and expressed as mean value and standard error of the mean.

stable than **TS-7a**). Here again, our DFT calculations suggested that **4a** should be the kinetic product and that **7a** should be the thermodynamic one. Moreover, we hypothesized that, in this case, the bulkiness of **10** precludes the addition from the more hindered face of **1**, as was observed for the generation of **6**.

***In Vitro* Antiproliferative Studies.** The *in vitro* antiproliferative activity of the 23 levoglucosenone-derived 1,2,3,-triazoles library was studied on the MDA-MB-231 cell line, which was originally derived from triple negative breast cancer (TNBC). This cell line lacks a wt allele at the *TP53* locus but retains a mutated one, allowing exclusive expression of the endogenous p53R280 K mutant protein. Upon treatment with each individual compound for 48 h, living cells were quantified using the MTT viability assay and normalized comparing with untreated cells (Figure 9). Compounds were initially tested at a 50 μM concentration, and only those that showed prominent cytotoxicity (less than 25% survival) were selected for further trials. We found that 12 compounds met this criteria (**4b−i**, **4l−m**, **7b**, and **7k**), supporting our initial hypothesis. To complete the preliminary characterization of the library, the remaining candidates were tested using a higher concentration (100 μM). Compounds **5a**, **5b**, **5c**, **5d**, and **5g** did not show a significant increase in cytotoxicity. Conversely, the other compounds tested showed an enhanced cytotoxicity at 100 μM and, in particular, for **4a**, **4j**, **7d**, and **7g**, a decrease in survival below 25% was observed (see Figure S1 in the Supporting Information).

Next, we studied the effect of the selected compounds at different concentrations (Figure 10 and Figure S2). All of the compounds tested showed a similar behavior, with GI50 (growth inhibitory 50) values ranging from 22.76 to 32.81 μM

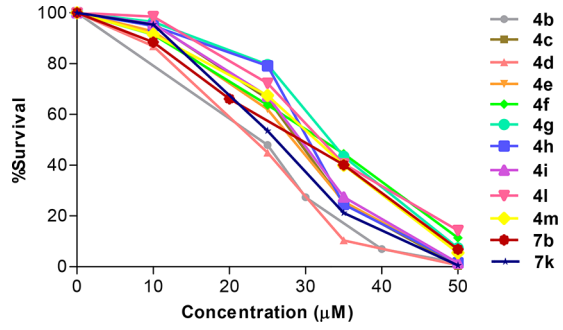

Figure 10. Characterization of the active concentration range for the levoglucosenone-derived 1,2,3,-triazoles that showed cytotoxicity on MDA-MB-231 breast cancer cells. Survival was normalized to control treatment (DMSO) and expressed as mean value.

(Table S1 in the Supporting Information). This cytotoxicity is comparable to that of PRIMA-1 (GI50 34 μM) on the same cell line in similar experimental conditions.[23] On the basis of the information collected in Figure 9 and Figure S1, some structure−activity relationships (SAR) could be established to detect the most influential molecular requirements for further trials. When assessing the influence of the stereochemistry at C-4, we noticed that the C-4-β-derivatives (**5a−d,g**) displayed a much lower activity than the corresponding C-4-α-analogues (**4a−d,g**), suggesting that the axial orientation of the triazolic fragment at C-4 plays a key role in terms of cytotoxicity. A similar trend was noted when analyzing the effect of the substitution pattern of the triazole (1,4 vs 2,4), being the former (**4**), more active than their corresponding isomers **7**, with the only exception of compound **7k**. However, the loss of activity when passing from **4** → **7** is less sharp than that noted for **4** → **5**, suggesting that the effect of the absolute configuration at C-4 is more influential on the cytotoxic activity. The analysis of the effect of the nature of substituents at C-4 in the triazoles **4** was less straightforward, as many structurally diverse compounds showed similar biological activities. However, we noticed that the moieties containing carbonyl groups or oxygen or sulfur substituted phenyl groups tend to afford better results. Finally, the need of the ketone group at C-2 was evidenced by the complete loss of cytotoxicity observed upon reduction with $NaBH_4$ of the most promising agents **4b** and **7b** (*vide infra*).

We then sought to understand if the observed cytotoxicity depends on the presence of mutant p53. To this end, we analyzed the effect of the selected compounds on MDA-MB-231 cells where p53R280 K was knocked down by shRNA expression. Cells where transduced with a plasmid expressing shp53 or control shRNA and selected. The mutant p53 knock down was confirmed by Western blot (Figure S3 in the Supporting Information). Survival assays were then performed upon treatment of transduced cells with different concentrations of each compound. We found a significant increase in survival upon mutant p53 knock down when cells were treated with compounds **4b** and **7b** (Figure 11 and Table S2). Collectively, our results identified novel compounds with cytotoxic activity against TNBC cells *in vitro*. This breast cancer subtype represents a clinical challenge since tumors are frequently resistant to current therapies. Therefore, the compounds identified in this work may provide leading molecules to further explore mechanisms to efficiently eliminate TNBC cells. In this regard, compounds **4b** and **7b** are of particular interest, since their cytotoxic effect was enhanced in the presence of mutant p53. Consequently, our

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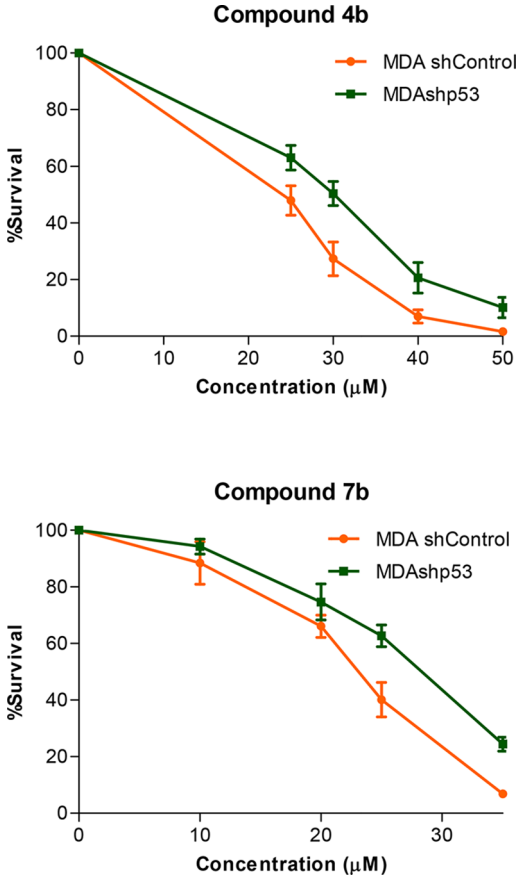

Figure 11. Cytotoxicity of compounds **4b** and **7b** is reduced upon mutant p53 silencing. Survival assays for the indicated compounds on MDA-MB-231 breast cancer cells transduced with shControl or shp53. Survival was normalized to DMSO control and expressed as mean value and standard error of the mean (sem).

results suggest that compounds **4b** and **7b** may show a higher selectivity for tumor cells expressing mutant p53, rather than for normal cells, which retain wt p53 expression.

CONCLUSIONS

In summary, we have reported the synthesis of chiral 1,2,3-triazoles derived from levoglucosenone, which in turn can be easily obtained from renewable feedstocks. The strategy relied on an aza-Michael addition of azide followed by Cu(I)-catalyzed 1,3-dipolar cycloadditions with terminal alkynes. Using *retro*-aza-Michael//aza-Michael cascade events, we could successfully explore the chemical space of these compounds by preparing the corresponding epimers at C-4 and the synthesis of the 2,4-disubstituted-1,2,3-triazole analogues as well. To the best of our knowledge, this is the first report on the preparation of 2,4-disubstituted 1,2,3-triazoles from the thermodynamic equilibration of the corresponding 1,4-disubstituted precursors following a *retro*-aza-Michael//aza-Michael path. The *in vitro* cytotoxic activity of all synthesized compounds was evaluated against TNBC cancer cell lines, and some of the tested ones showed a satisfactory antitumor activity. Some clear structure−activity relationships could be drawn, and the cytotoxicity dependence on the presence of mutant p53 was also observed. This work demonstrates the possibility of obtaining new and promising antitumor leading molecules from biomass derivatives, providing a sustainable strategy for the transformation of urban and industrial wastes into valuable chemicals.

EXPERIMENTAL SECTION

All reagents and solvents were used directly as purchased or purified according to standard procedures. Analytical thin-layer chromatography was carried out using commercial silica gel plates and visualization was effected with a short wavelength UV light (254 nm) and a *p*-anysaldehyde solution (2.5 mL of *p*-anysaldehyde, 2.5 mL of H_2SO_4, 0.25 mL of AcOH, and 95 mL of EtOH) with subsequent heating. Column chromatography was performed with silica gel 60 H, and the samples slurry packed and run under low pressure of nitrogen using mixtures of hexane and ethyl acetate. NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C with $CDCl_3$ as a solvent and $(CH_3)_4Si$ (^{1}H) or $CDCl_3$ (^{13}C, 76.9 ppm) as an internal standards. Chemical shifts (δ) are reported in parts per million (ppm), and splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are recorded in hertz (Hz). Isomeric ratios were determined by ^{1}H NMR analysis. The structure of the products were determined by a combination of spectroscopic methods such as IR, 1D and 2D NMR (including NOE, DEPT, COSY, HSQC, and HMBC experiments) and HRMS. Infrared spectra were recorded using sodium chloride plates pellets. Absorbance frequencies are recorded in reciprocal centimeters (cm^{-1}). High-resolution mass spectra (HRMS) were obtained on a TOF-Q LC-MS spectrometer. Levoglucosenone (**1**) was obtained from the microwave-assisted pyrolysis of cellulose following our previously reported procedure.[3]

General Procedure for the Synthesis of C-4-α-1,4-Disubstituted-1,2,3-triazolyl Derivatives (4). To a solution of **1** (35 mg, 0.28 mmol) in acetic acid (1 mL) were added sodium azide (62 mg, 0.95 mmol) and NEt_3 (6 μL, 0.04 mmol). The mixture was stirred at room temperature for 10 min, and then water (5 mL) was added. The aqueous phase was extracted with AcOEt (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was dissolved in a 50:50 mixture of CH_2Cl_2/H_2O (1.0 mL), and the corresponding alkyne (0.36 mmol), sodium ascorbate (22 mg, 0.11 mmol), and $CuSO_4{\cdot}5H_2O$ (9.8 mg, 0.039 mmol) were added in that order. The mixture was stirred at room temperature for 1 h, and then water (5 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL) and then AcOEt (2 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (hex/AcOEt 60:40 → 0:100, gradient 5%) to afford the 1,4-disubstituted 1,2,3-triazoles **4a−m** in the yields indicated in Table 1. *Caution should be exercised when using azides!*

Compound ***4a:*** 65.3 mg, 86% yield; white crystalline solid; mp 142−143 °C (CH_2Cl_2/hex); $[\alpha]_D^{24}$ −250.4 (*c* 0.89, $CHCl_3$); IR (KBr) ν_{max} 3125, 2914, 1741 (C═O), 1481, 1115, 974, 912, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1H, H-7), 7.80−7.73 (m, 2H, arom), 7.42−7.24 (m, 3H, arom), 5.36 (d, J = 7.8 Hz, 1H, H-4), 5.28 (s, 1H, H-1), 4.87 (bd, J = 4.8 Hz, 1H, H-5), 4.20 (dd, J = 8.2 Hz, J = 1.0 Hz, 1H, H-6_{endo}), 4.10 (dd, J = 8.4 Hz, J = 5.4 Hz, 1H, H-6_{exo}), 3.26 (dd, J = 17.4 Hz, J = 8.1 Hz, 1H, H-3_{ax}), 2.70 (ddd, J = 17.7 Hz, J ∼ 1.1 Hz J ∼ 1.1 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 196.1 (CH, C-2), 148.4 (C, C-8), 129.9 (C, arom), 128.8 (CH, 2C, arom), 128.4 (CH, arom), 125.7 (CH, 2C, arom), 118.1 (CH, C-7), 101.4 (CH, C-1), 76.1 (CH, C-5), 66.3 (CH_2, C-6), 60.2 (CH, C-4), 36.9 (CH_2, C-3); HRMS calcd for $C_{14}H_{13}N_3O_3Na$ (M + Na) 294.0849, found 294.0843.

Compound ***4b:*** 53.9 mg, 76% yield; white crystalline solid; mp 145−146 °C (hex/AcOEt); $[\alpha]_D^{34}$ −170.0 (*c* 0.98, AcOEt); IR (KBr) ν_{max} 3165, 2957, 1744 (C═O), 1719 (C═O), 1543, 1441, 1234, 1117, 914 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H, H-7), 5.47 (d, J = 7.8 Hz, 1H, H-4), 5.34 (s, 1H, H-1), 4.92 (d, J = 5.1 Hz, 1H, H-5), 4.28 (dd, J = 8.5 Hz, J = 0.7 Hz, 1H, H-6_{endo}), 4.19 (dd, J = 8.5 Hz, J = 5.3 Hz, 1H, H-6_{exo}), 3.97 (s, 3H, H-10), 3.35 (dd, J = 17.6 Hz, J = 7.9 Hz, 1H, H-3_{ax}), 2.73 (dd, J = 17.6 Hz, J = 1.1 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 195.3 (C, C-2), 160.6 (C, C-9), 140.5 (C, C-8),

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126.2 (CH, C-7), 101.4 (CH, C-1), 75.7 (CH, C-5), 66.2 (CH_2, C-6), 60.5 (CH, C-4), 52.2 (CH_3, C-10), 36.7 (CH_2, C-3); HRMS calcd for $C_{10}H_{11}N_3O_5Na$ (M + Na) 276.0591, found 276.0590.

Compound ***4c:*** 70.1 mg, 83% yield; yellowish solid; mp 157−158 °C (hex/AcOEt); $[\alpha]_D^{20}$ −213.2 (*c* 1.18, CH_2Cl_2); IR (KBr) ν_{max} 1744 (C═O), 1616, 1560, 1491, 1415, 1250, 1112, 1026, 966, 916 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (s, 1H, H-7), 7.76 (d, *J* = 8.8 Hz, 2H, arom), 6.96 (d, *J* = 8.4 Hz, 2H, arom), 5.42 (d, *J* = 8.1 Hz, 1H, H-4), 5.34 (s, 1H, H-1), 4.93 (d, *J* = 4.5 Hz, 1H, H-5), 4.26 (dd, *J* = 8.3 Hz, *J* = 0.7 Hz, 1H, H-6_{endo}), 4.16 (dd, *J* = 8.1 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.85 (s, 3H, H-9), 3.32 (dd, *J* = 17.5 Hz, *J* = 8.0 Hz, 1H, H-3_{ax}), 2.76 (d, *J* = 17.1 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 196.2 (C, C-2), 159.7 (C, arom), 148.4 (C, C-8), 127.0 (CH, arom), 122.7 (C, arom), 117.3 (CH, C-7), 114.2 (CH, arom), 101.4 (CH, C-1), 76.1 (CH, C-5), 66.3 (CH_2, C-6), 60.2 (CH, C-4), 55.2 (CH_3, C-9), 36.9 (CH_2, C-3); HRMS calcd for $C_{15}H_{15}N_3O_4Na$ (M + Na) 324.0955, found 324.0953.

Compound ***4d:*** 66.3 mg, 77% yield; white solid; mp 81−82 °C (hex/AcOEt); $[\alpha]_D^{21}$ −177.6 (*c* 1.08, $CHCl_3$); IR (KBr) ν_{max} 2955, 2920, 2851, 1741 (C═O), 1113, 968, 914, 878, 661 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.41 (s, 1H, H-7), 5.34 (d, *J* = 7.8 Hz, 1H, H-4), 5.28 (s, 1H, H-1), 4.85 (d, *J* = 4.8 Hz, 1H, H-5), 4.23 (dd, *J* = 8.4 Hz, *J* = 0.9 Hz, 1H, H-6_{endo}), 4.11 (ddd, *J* = 7.9 Hz, *J* = 5.6 Hz, *J* = 1.1 Hz, 1H, H-6_{exo}), 3.27 (dd, *J* = 17.4 Hz, *J* = 8.1 Hz, 1H, H-3_{ax}), 2.73−2.61 (m, 3H, H-3_{eq} and H-9*), 1.71−1.56 (m, 2H, H-10*), 1.39−1.16 (m, 10H, H-11*, H-12*, H-13*, H-14*, and H-15*), 0.91−0.78 (m, 3H, H-16); ^{13}C NMR ($CDCl_3$) δ 196.4 (C, C-2), 149.1 (C, C-8), 119.1 (CH, C-7), 101.3 (CH, C-1), 76.1 (CH, C-5), 66.2 (CH_2, C-6), 59.9 (CH, C-4), 36.8 (CH_2, C-3), 31.7 (CH_2, C-9*), 29.2 (CH_2, C-10*), 29.1 (CH_2, 2C, C-11* and C-12*), 29.0 (CH_2, C-13*), 25.5 (CH_2, C-14*), 22.5 (CH_2, C-15*), 13.9 (CH_3, C-16); HRMS calcd for $C_{16}H_{25}N_3O_3Na$ (M + Na) 330.1788, found 330.1803.

Compound ***4e:*** 47.9 mg, 64% yield; yellowish oil; $[\alpha]_D^{25}$ −151.4 (*c* 1.08, $CHCl_3$); IR (film) ν_{max} 2968, 1743 (C═O), 1230, 113, 1033, 970, 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.78 (s, 1H, H-7), 5.39 (d, *J* = 8.0 Hz, 1H, H-4), 5.31 (s, 1H, H-1), 5.21 (s, 2H, H-9), 4.90 (d, *J* = 4.7 Hz, 1H, H-5), 4.26 (dd, *J* = 8.4 Hz, *J* = 1.0 Hz, 1H, H-6_{endo}), 4.16 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.31 (dd, *J* = 17.6 Hz, *J* = 8.0 Hz, 1H, H-3_{ax}), 2.73 (ddd, *J* = 17.6 Hz, *J* = 1.2 Hz, *J* = 0.9 Hz, 1H, H-3_{eq}), 2.09 (s, 3H, H-11); ^{13}C NMR ($CDCl_3$) δ 195.8 (C, C-2), 170.6 (C, C-10), 143.4 (C, C-8), 122.2 (CH, C-7), 101.3 (CH, C-1), 75.9 (CH, C-5), 66.2 (CH_2, C-6), 60.1 (CH, C-4), 57.3 (CH_2, C-9), 36.7 (CH_2, C-3), 20.7 (CH_3, C-11); HRMS calcd for $C_{11}H_{13}N_3O_5Na$ (M + Na) 290.0747, found 290.0758.

Compound ***4f:*** 53.4 mg, 65% yield; colorless oil; $[\alpha]_D^{21}$ −153.8 (*c* 0.77, $CHCl_3$); IR (film) ν_{max} 1738 (C═O), 1732 (C═O), 1367, 1232, 1113, 1049, 1026, 968, 910, 879 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68 (s, 1H, H-7), 6.65 (d, *J* = 16.0 Hz, 1H, H-9), 6.50 (dt, *J* = 16.0 Hz, *J* = 6.0 Hz, 1H, H-10), 5.38 (d, *J* = 8.1 Hz, 1H, H-4), 5.31 (s, 1H, H-1), 4.88 (d, *J* = 5.1 Hz, 1H, H-5), 4.73 (dd, *J* = 5.9 Hz, *J* = 1.1 Hz, 2H, H-11), 4.26 (dd, *J* = 8.4 Hz, *J* = 0.9 Hz, 1H, H-6_{endo}), 4.15 (dd, *J* = 8.4 Hz, *J* = 5.1 Hz, 1H, H-6_{exo}), 3.31 (dd, *J* = 17.6 Hz, *J* = 8.0 Hz, 1H, H-3_{ax}), 2.69 (ddd, *J* = 17.5 Hz, *J* = 1.1 Hz, *J* = 1.0 Hz, 1H, H-3_{eq}), 2.11 (s, 3H, H-13); ^{13}C NMR ($CDCl_3$) δ 196.0 (C, C-2), 170.6 (C, C-12), 145.6 (C, C-8), 126.2 (CH, C-10), 121.3 (CH, C-9), 119.1 (CH, C-7), 101.3 (CH, C-1), 76.0 (CH, C-5), 66.2 (CH_2, C-6), 64.2 (CH_2, C-11), 60.1 (CH, C-4), 36.8 (CH_2, C-3), 20.8 (CH_3, C-13); HRMS calcd for $C_{13}H_{15}N_3O_5Na$ (M + Na) 316.0904, found 316.0918.

Compound ***4g:*** 59.9 mg, 71% yield; yellowish glass; $[\alpha]_D^{22}$ −97.0 (*c* 0.99, CH_3OH); IR (film) ν_{max} 3346, 3161, 2968, 1744 (C═O), 1113, 1093, 1051, 972, 916 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50 (s, 1H, H-7), 7.43−7.23 (m, 5H, arom), 5.96 (d, *J* = 3.3 Hz, 1H, H-9), 5.25 (d, *J* = 8.1 Hz, 1H, H-4), 5.21 (s, 1H, H-1), 4.78 (s, 1H, H-5), 4.15 (d, *J* = 8.4 Hz, 1H, H-6_{endo}), 4.03 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.83 (s, 1H, −OH), 3.18 (dd, *J* = 17.6 Hz, *J* = 8.0 Hz, 1H, H-3_{ax}), 2.65 (d, *J* = 17.6 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 196.1 (C, C-2), 151.7 (C, C-8), 141.7 (C, arom), 128.5 (CH, arom), 127.9 (CH, arom), 126.3 (CH, arom), 120.0 (CH, C-7), 101.1 (CH, C-1), 75.8 (CH, C-5), 68.9 (CH, C-9), 66.1 (CH_2, C-6), 60.0 (CH, C-4), 36.4 (CH_2, C-3); HRMS calcd for $C_{15}H_{15}N_3O_4Na$ (M + Na) 324.0955, found 324.0965.

Compound ***4h:*** 54.8 mg, 65% yield; white solid; mp 130−131 °C (hex/AcOEt); $[\alpha]_D^{26}$ −108.7 (*c* 1.07, CH_3OH); IR (KBr) ν_{max} 2963, 1746 (C═O), 1599, 1494, 1236, 1113, 970, 756 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.81 (s, 1H, H-7), 7.35−7.25 (m, 2H, arom), 7.04−6.94 (m, 3H, arom), 5.37 (d, *J* = 6.9 Hz, 1H, H-4), 5.30 (s, 1H, H-1), 5.19 (s, 2H, H-9), 4.89 (d, *J* = 3.0 Hz, 1H, H-5), 4.24 (d, *J* = 8.1 Hz, 1H, H-6_{endo}), 4.17−4.10 (m, 1H, H-6_{exo}), 3.29 (dd, *J* = 17.4 Hz, *J* = 6.3 Hz, 1H, H-3_{ax}), 2.73 (d, *J* = 17.1 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 196.0 (C, C-2), 158.0 (C, arom), 144.6 (C, C-8), 129.4 (CH, arom), 121.6 (CH, C-7), 121.2 (C, arom), 114.5 (CH, arom), 101.2 (CH, C-1), 75.9 (CH, C-5), 66.1 (CH_2, C-6), 61.5 (CH_2, C-9), 60.0 (CH, C-4), 36.6 (CH_2, C-3); HRMS calcd for $C_{15}H_{15}N_3O_4Na$ (M + Na) 324.0955, found 324.0966.

Compound ***4i:*** 77.3 mg, 87% yield; yellowish glass; $[\alpha]_D^{23}$ −149.3 (*c* 1.02, $CHCl_3$); IR (film) ν_{max} 2922, 1745 (C═O), 1481, 1439, 1227, 1113, 1047, 968, 910, 879 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (s, 1H, H-7), 7.35−7.15 (m, 5H, arom), 5.28 (d, *J* = 8.1 Hz, 1H, H-4), 5.23 (s, 1H, H-1), 4.79 (d, *J* = 5.1 Hz, 1H, H-5), 4.23−4.17 (m, 3H, H-6_{endo} and H-9), 4.07 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.23 (dd, *J* = 17.6 Hz, *J* = 7.9 Hz, 1H, H-3_{ax}), 2.60 (ddd, *J* = 17.4 Hz, *J* = 1.2 Hz, *J* = 1.0 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 196.0 (C, C-2), 145.1 (C, C-8), 134.8 (C, arom), 130.0 (CH, arom), 128.8 (CH, arom), 126.6 (CH, arom), 120.7 (CH, C-7), 101.1 (CH, C-1), 75.8 (CH, C-5), 66.0 (CH_2, C-6), 59.8 (CH, C-4), 36.5 (CH_2, C-3), 28.9 (CH_2, C-9); HRMS calcd for $C_{15}H_{15}N_3O_3SNa$ (M + Na) 340.0726, found 340.0728.

Compound ***4j:*** 42.9 mg, 51% yield; yellowish glass; $[\alpha]_D^{24}$ −176.8 (*c* 1.11, $CHCl_3$); IR (film) ν_{max} 3395, 2922, 1745, 1602, 1504, 1315, 1115, 970, 910, 879 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.63 (s, 1H, H-7), 7.23−7.14 (m, 2H, arom), 6.78−6.63 (m, 3H, arom), 5.31 (d, *J* = 8.1 Hz, 1H, H-4), 5.27 (s, 1H, H-1), 4.85 (d, *J* = 4.8 Hz, 1H, H-5), 4.44 (s, 2H, H-9), 4.20 (dd, *J* = 8.4 Hz, *J* = 1.2 Hz, 1H, H-6_{endo}), 4.11 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.25 (dd, *J* = 17.6 Hz, *J* = 7.9 Hz, 1H, H-3_{ax}), 2.69 (ddd, *J* = 17.5 Hz, *J* = 1.2 Hz, *J* = 1.0 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 196.0 (C, C-2), 147.4 (C, arom), 146.7 (C, C-8), 129.2 (CH, arom), 120.3 (CH, C-7), 118.0 (CH, arom), 113.1 (CH, arom), 101.2 (CH, C-1), 75.9 (CH, C-5), 66.1 (CH_2, C-6), 60.1 (CH, C-4), 39.7 (CH_2, C-9), 36.6 (CH_2, C-3); HRMS calcd for $C_{15}H_{16}N_4O_3Na$ (M + Na) 323.1115, found 323.1120.

Compound ***4k:*** 76.1 mg, 82% yield; white solid; mp 104−105 °C (hex/AcOEt); $[\alpha]_D^{21}$ −158.8 (*c* 1.10, $CHCl_3$); IR (KBr) ν_{max} 1745 (C═O), 1508, 1238, 1113, 1040, 1005, 968, 881, 825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.79 (s, 1H, H-7), 6.96−6.90 (m, 2H, arom), 6.87−6.81 (m, 2H, arom), 5.38 (d, *J* = 8.1 Hz, 1H, H-4), 5.31 (s, 1H, H-1), 5.14 (s, 2H, H-9), 4.89 (d, *J* = 4.5 Hz, 1H, H-5), 4.24 (dd, *J* = 8.4 Hz, *J* = 0.9 Hz, 1H, H-6_{endo}), 4.14 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.77 (s, 3H, H-10), 3.29 (dd, *J* = 17.6 Hz, *J* = 7.9 Hz, 1H, H-3_{ax}), 2.73 (ddd, *J* = 17.6 Hz, *J* = 1.2 Hz, *J* = 1.0 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 195.9 (C, C-2), 154.2 (C, arom), 152.2 (C, arom), 145.0 (C, C-8), 121.4 (CH, C-7), 115.8 (CH, arom), 114.6 (CH, arom), 101.3 (CH, C-1), 76.0 (CH, C-5), 66.2 (CH_2, C-6), 62.5 (CH_2, C-9), 60.2 (CH, C-4), 55.6 (CH_3, $-OCH_3$), 36.7 (CH_2, C-3); HRMS calcd for $C_{16}H_{17}N_3O_5Na$ (M + Na) 354.1060, found 354.1065.

Compound ***4l:*** 81.4 mg, 84% yield; yellowish glass; $[\alpha]_D^{21}$ −152.5 (*c* 1.02, $CHCl_3$); IR (film) ν_{max} 1745 (C═O), 1607, 1524, 1352, 1279, 1250, 1113, 879, 746 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.92 (s, 1H, H-7), 7.83 (dd, *J* = 8.1 Hz, *J* = 1.8 Hz, 1H, arom), 7.59−7.52 (m, 1H, arom), 7.32 (dd, *J* = 8.5 Hz, *J* = 0.7 Hz, 1H, arom), 7.11−7.03 (m, 1H, arom), 5.38 (d, *J* = 7.8 Hz, 1H, H-4), 5.34 (s, 2H, H-9), 5.29 (s, 1H, H-1), 4.93 (d, *J* = 4.8 Hz, 1H, H-5), 4.27 (dd, *J* = 8.6 Hz, *J* = 1.0 Hz, 1H, H-6_{endo}), 4.13 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 1H, H-6_{exo}), 3.30 (dd, *J* = 17.6 Hz, *J* = 7.9 Hz, 1H, H-3_{ax}), 2.80 (ddd, *J* = 17.7 Hz, *J* = 1.2 Hz, *J* = 1.0 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 195.8 (C, C-2), 151.3 (C, arom), 143.4 (C, C-8), 140.0 (C, arom), 134.2 (CH, arom), 125.5 (CH, arom), 122.2 (CH, C-7), 121.1 (CH, arom), 115.4 (CH, arom), 101.2 (CH, C-1), 75.9 (CH, C-5), 66.1 (CH_2, C-6), 63.4 (CH_2, C-9), 60.1 (CH, C-4), 36.5 (CH_2, C-3); HRMS calcd for $C_{15}H_{14}N_4O_6Na$ (M + Na) 369.0806, found 369.0818.

Compound ***4m:*** 64.9 mg, 70% yield; yellowish oil; $[\alpha]_D^{20}$ −166.9 (*c* 0.99, $CHCl_3$); IR (film) ν_{max} 1744 (C═O), 1491, 1227, 1113, 1045,

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1001, 968, 910, 879 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (s, 1H, H-7), 7.26−7.22 (m, 2H, arom), 7.10−7.07 (m, 2H, arom), 5.29 (d, J = 8.1 Hz, 1H, H-4), 5.26 (s, 1H, H-1), 4.81 (d, J = 4.8 Hz, 1H, H-5), 4.20 (dd, J = 8.4 Hz, J = 0.9 Hz, 1H, H-6_{endo}), 4.15 (s, 2H, H-9), 4.11 (dd, J = 8.4 Hz, J = 5.4 Hz, 1H, H-6_{exo}), 3.23 (dd, J = 17.4 Hz, J = 8.1 Hz, 1H, H-3_{ax}), 2.60 (ddd, J = 17.4 Hz, J = 1.2 Hz, J = 1.0 Hz, 1H, H-3_{eq}), 2.32 (s, 3H, H-10); ^{13}C NMR ($CDCl_3$) δ 195.7 (C, C-2), 145.6 (C, C-8), 137.2 (C, arom), 131.2 (CH, arom), 131.1 (C, arom), 129.7 (CH, arom), 120.6 (CH, C-7), 101.3 (CH, C-1), 76.0 (CH, C-5), 66.1 (CH_2, C-6), 60.0 (CH, C-4), 36.7 (CH_2, C-3), 29.8 (CH_2, C-9), 20.9 (CH_3, —CH_3); HRMS calcd for $C_{16}H_{17}N_3O_3SNa$ (M + Na) 354.0883, found 354.0890.

General Procedure for the Synthesis of C-4-β-1,4-Disubstituted-1,2,3-triazolyl Derivatives (5). To a solution of **1** (35 mg, 0.28 mmol) in acetic acid (1 mL) were added sodium azide (62 mg, 0.95 mmol) and NEt_3 (6 μL, 0.04 mmol). The mixture was stirred at room temperature for 10 min, and then water (5 mL) was added. The aqueous phase was extracted with AcOEt (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was dissolved in CH_2Cl_2 (0.5 mL), and NEt_3 (3.9 μL, 0.027 mmol) was added. After the mixture stirred for 3 h, water (0.5 mL), the corresponding alkyne (0.36 mmol), sodium ascorbate (22 mg, 0.11 mmol), and $CuSO_4 \cdot 5H_2O$ (9.8 mg, 0.039 mmol) were added in that order. The mixture was stirred at room temperature for 1 h, and then water (5 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL) and then AcOEt (2 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (hex/AcOEt 70:30 → 0:100, gradient 3%) to afford the corresponding mixture of compounds **4** and **5** in the yield and selectivity indicated in Table 3. *Caution should be exercised when using azides!*

Compound ***5a:*** 43.4 mg, 57% yield; white crystalline solid; mp 168−169 °C (DMSO/EtOH); $[\alpha]_D^{27}$ −9.27 (*c* 0.27, CH_3OH); IR (KBr) ν_{max} 3082, 2920, 1734 (C═O), 1182, 1109, 1070, 912 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86−7.77 (m, 3H, H-7 and arom), 7.49−7.34 (m, 3H, arom), 5.27 (s, 1H, H-1), 5.24−5.15 (m, 1H, H-4), 5.06 (broad s, 1H, H-5), 4.64 (d, J = 8.7 Hz, 1H, H-6_{endo}), 4.01 (dd, J = 8.4 Hz, J = 5.1 Hz, 1H, H-6_{exo}), 3.57 (dd, J = 15.6 Hz, J = 11.7 Hz, 1H, H-3_{ax}), 3.04 (dd, J = 15.9 Hz, J = 6.3 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 195.4 (C, C-2), 148.1 (C, C-8), 129.6 (C, arom), 128.9 (CH, 2C, arom), 128.6 (CH, arom), 125.7 (CH, 2C, arom), 119.0 (CH, C-7), 100.4 (CH, C-1), 74.9 (CH, C-5), 64.8 (CH_2, C-6), 58.3 (CH, C-4), 37.2 (CH_2, C-3); HRMS calcd for $C_{14}H_{13}N_3O_3Na$ (M + Na) 294.0849, found 294.0848.

Compound ***5b:*** 41.3 mg, 58% yield; white crystalline solid; mp 153−154 °C (hex/AcOEt); $[\alpha]_D^{28}$ −72,0 (*c* 0.41, AcOEt); IR (KBr) ν_{max} 3069, 2980, 2930, 1722 (C═O), 1236, 1111, 1043, 912 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.20 (s, 1H, H-7), 5.30−5.18 (m, 2H, H-1 and H-4), 5.06 (broad s, 1H, H-5), 4.55 (d, J = 8.7 Hz, H-6_{endo}), 4.04−3.94 (m, 4H, H-6_{exo} and H-10), 3.51 (dd, J = 15.7 Hz, J = 11.7 Hz, 1H, H-3_{ax}), 3.04 (dd, J = 15.7 Hz, J = 6.5 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 194.6 (C, C-2), 160.5 (C, C-9), 140.3 (C, C-8), 127.0 (CH, C-7), 100.3 (CH, C-1), 74.7 (CH, C-5), 64.6 (CH_2, C-6), 58.5 (CH, C-4), 52.4 (CH_3, C-10), 37.0 (CH_2, C-3); HRMS calcd for $C_{10}H_{11}N_3O_5Na$ (M + Na) 276.0591, found 276.0587.

Compound ***5c:*** 50.6 mg, 60% yield; white solid; mp 155−156 °C; $[\alpha]_D^{28}$ −2.5 (*c* 0.95, CH_3OH); IR (KBr) ν_{max} 3422, 2920, 2851, 1734 (C═O), 1616, 1499, 1248, 1177, 922 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.79−7.71 (m, 3H, H-7 and arom), 7.00−6.94 (m 2H, arom), 5.26 (s, 1H, H-1), 5.23−5.12 (m, 1H, H-4), 5.04 (s, 1H, H-5), 4.63 (d, J = 8.7 Hz, 1H, H-6_{endo}), 4.00 (dd, J = 8.7 Hz, J = 5.1 Hz, 1H, H-6_{exo}), 3.85 (s, 3H, H-9), 3.57 (dd, J = 15.6 Hz, J = 11.7 Hz, 1H, H-3_{ax}), 3.03 (dd, J = 15.9 Hz, J = 6.6 Hz, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 195.5 (C, C-2), 159.9 (C, arom), 148.0 (C, C-8), 127.0 (CH, C-7), 122.3 (C, arom), 118.2 (CH, arom), 114.3 (CH, arom), 100.4 (CH, C-1), 74.9 (CH, C-5), 64.8 (CH_2, C-6), 58.2 (CH, C-4), 55.2 (CH_3, C-9), 37.2 (CH_2, C-3); HRMS calcd for $C_{15}H_{15}N_3O_4Na$ (M + Na) 324.0955, found 324.0956.

Compound ***5d:*** 48.9 mg, 57% yield; white solid; mp 101−102 °C; $[\alpha]_D^{29}$ −55.2 (*c* 0.76, $CHCl_3$); IR (KBr) ν_{max} 3117, 2965, 2850, 1734 (C═O), 1466, 1124, 1109, 1049, 968, 887 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34 (s, 1H, H-7), 5.23 (s, 1H, H-1), 5.15−5.04 (m, 1H, H-4), 4.98 (bs, 1H, H-5), 4.58 (dd, J = 8.6 Hz, J = 0.8 Hz, 1H, H-6_{endo}), 3.97 (dd, J = 8.3 Hz, J = 5.0 Hz, 1H, H-6_{exo}), 3.50 (dd, J = 15.9 Hz, J = 11.6 Hz, 1H, H-3_{ax}), 2.97 (dddd, J = 15.9 Hz, J = 6.7 Hz, J = 1.8 Hz, J = 1.1 Hz, 1H, H-3_{eq}), 2.71 (t, J = 7.5 Hz, 2H, H-9), 1.73−1.60 (m, 2H, H-10*), 1.40−1.22 (m, 10H, H-11*, H-12*, H-13*, H-14*, and H-15*), 0.92−0.84 (m, 3H, H-16); ^{13}C NMR ($CDCl_3$) δ 195.7 (C, C-2), 148.9 (C, C-8), 120.0 (CH, C-7), 100.3 (CH, C-1), 74.9 (CH, C-5), 64.7 (CH_2, C-6), 57.9 (CH, C-4), 37.2 (CH_2, C-3), 31.7 (CH_2, C-9*), 29.2 (CH_2, 3C, C-10*, C-11*, and C-12*), 29.1 (CH_2, C-13*), 25.4 (CH_2, C-14*), 22.5 (CH_2, C-15*), 14.0 (CH_3, C-16); HRMS calcd for $C_{16}H_{25}N_3O_3Na$ (M + Na) 330.1788, found 330.1790.

Compound ***5g:*** 38.4 mg, 46% yield; yellowish oil; $[\alpha]_D^{33}$ −49.7 (*c* 0.73, CH_2Cl_2); IR (film) ν_{max} 3361, 2924, 1732 (C═O), 1456, 1261, 1068, 926 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.49−7.29 (m, 6H, H-7 and arom), 6.04 (s, 1H, H-9), 5.22 (s, 1H, H-1), 5.10−5.00 (m, 1H, H-4), 4.95 (bs, 1H, H-5), 4.55 (ddd, J = 8.7 Hz, J = 2.7 Hz, J = 0.6 Hz, 1H, H-6_{endo}), 4.00−3.91 (m, 1H, H-6_{exo}), 3.48 (dd, J = 15.9 Hz, J = 9.5 Hz, 1H, H-3_{ax}), 3.10 (sa, 1H, —OH), 2.98−2.88 (m, 1H, H-3_{eq}); ^{13}C NMR ($CDCl_3$) δ 195.4 (C, C-2), 151.7 (C, C-8), 141.4 (C, arom), 128.7 (CH, arom), 128.2 (CH, arom), 126.2 (CH, arom), 120.9 (CH, C-7), 100.3 (CH, C-1), 74.8 (CH, C-5), 69.1 (CH, C-9), 64.7 (CH_2, C-6), 58.2 (CH, C-4), 37.2 (CH_2, C-3); HRMS calcd for $C_{15}H_{15}N_3O_4Na$ (M + Na) 324.0955, found 324.0957.

General Procedure for the Synthesis of C-4-α-2,4-Disubstituted-1,2,3-triazolyl Derivatives (7). To a solution of pure compound **4** (0.12 mmol) in $CHCl_3$ (0.3 mL) was added NEt_3 (16.7 μL, 0.12 mmol). The mixture was stirred at 70 °C for 12 h in a Hach tube. The solvent was evaporated under reduced pressure, and the crude material was purified by flash chromatography (hex/AcOEt 80:20 → 50:50, gradient 2%) to afford the corresponding compound **7** in the yield indicated in Table 5.

Compound ***7a:*** 28.1 mg, 86% yield; yellowish glass; $[\alpha]_D^{27}$ −283.3 (*c* 0.73, $CHCl_3$); IR (film) ν_{max} 2972, 2914, 1748 (C═O), 1475, 1117, 978, 908, 883 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.89 (s, 1H, H-7), 7.82−7.71 (m, 2H, arom), 7.50−7.30 (m, 3H, arom), 5.24 (s, 1H, H-1), 5.19−5.08 (m, 2H, H-4 and H-5), 4.20 (dd, J = 8.1 Hz, J = 0.9 Hz, 1H, H-6_{endo}), 4.08 (dd, J = 8.2 Hz, J = 5.3 Hz, 1H, H-6_{exo}), 3.35 (d, J = 17.1 Hz, 1H, H-3_{eq}), 3.12 (dd, J = 17.2 Hz, J = 7.3 Hz, 1H, H-3_{ax}); ^{13}C NMR ($CDCl_3$) δ 195.8 (C, C-2), 148.2 (C, C-8), 131.4 (CH, C-7), 129.8 (C, arom), 128.7 (CH, 2C, arom), 128.6 (CH, arom), 125.8 (CH, 2C, arom), 101.4 (CH, C-1), 76.1 (CH, C-5), 65.6 (CH_2, C-6), 63.2 (CH, C-4), 34.9 (CH_2, C-3); HRMS calcd for $C_{14}H_{13}N_3O_3Na$ (M + Na) 294.0849, found 294.0849.

Compound ***7b:*** 26.4 mg, 87% yield; colorless oil; $[\alpha]_D^{23}$ −239.4 (*c* 1.08, $CHCl_3$); IR (film) ν_{max} 2961, 2918, 1740 (C═O), 1512, 1319, 1234, 1115, 1034 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.09 (s, 1H, H-7), 5.25−5.13 (m, 2H, H-1 and H-4), 5.08 (bs, 1H, H-5), 4.20 (dd, J = 8.2 Hz, J = 1.0 Hz, 1H, H-6_{endo}), 4.09 (dd, J = 8.4 Hz, J = 5.4 Hz, 1H, H-6_{exo}), 3.95 (s, 3H, H-10), 3.30 (dddd, J = 17.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, J = 1.4 Hz, 1H, H-3_{eq}), 3.13 (dd, J = 17.4 Hz, J = 7.3 Hz, 1H, H-3_{ax}); ^{13}C NMR ($CDCl_3$) δ 195.0 (C, C-2), 160.6 (C, C-9), 140.4 (C, C-8), 137.0 (CH, C-7), 101.3 (CH, C-1), 75.9 (CH, C-5), 65.6 (CH_2, C-6), 64.1 (CH, C-4), 52.3 (CH_3, C-10), 34.7 (CH_2, C-3); HRMS calcd for $C_{10}H_{12}N_3O_5$ (M + H) 254.0772, found 254.0770.

Compound ***7d:*** 26.2 mg, 71% yield; yellowish oil; $[\alpha]_D^{31}$ −186.1 (*c* 1.12, $CHCl_3$); IR (film) ν_{max} 2955, 2855, 1748 (C═O), 1117, 908, 883 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.41 (s, 1H, H-7), 5.22 (s, 1H, H-1), 5.08−5.03 (m, 2H, H-4 and H-5), 4.17 (dd, J = 8.3 Hz, J = 1.1 Hz, 1H, H-6_{endo}), 4.07 (dd, J = 8.1 Hz, J = 4.9 Hz, 1H, H-6_{exo}), 3.27 (ddd, J = 17.3 Hz, J = 3.2 Hz, J = 1.4 Hz, 1H, H-3_{eq}), 3.07 (dd, J = 17.3 Hz, J = 7.1 Hz, 1H, H-3_{ax}), 2.66 (t, J = 7.5 Hz, 2H, H-9), 1.72−1.58 (m, 2H, H-10*), 1.41−1.20 (m, 10H, H-11*, H-12*, H-13*, H-14* and H-15*), 0.92−0.84 (m, 3H, H-16); ^{13}C NMR ($CDCl_3$) δ 196.0 (C, C-2), 149.4 (C, C-8), 133.1 (CH, C-7), 101.3 (CH, C-1), 76.1 (CH, C-5), 65.6 (CH_2, C-6), 62.7 (CH, C-4), 35.0 (CH_2, C-3), 31.7 (CH_2, C-9*), 29.1 (CH_2, 3C, C-10*, C-11* and C-12*), 29.0 (CH_2, C-13*), 25.4

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(CH_2, C-14*), 22.5 (CH_2, C-15*), 14.0 (CH_3, C-16); HRMS calcd for $C_{16}H_{25}N_3O_3Na$ (M + Na) 330.1788, found 330.1792.

Compound **7g**: 30.1 mg, 83% yield; white oil; $[\alpha]_D^{29}$ −168.6 (*c* 1.01, $CHCl_3$); IR (film) ν_{max} 3418, 3402, 2918, 1788 (C═O), 1406, 1305, 1115, 1022, 968, 881 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45 (s, 1H, H-7), 7.44−7.27 (m, 5H, arom), 5.97 (s, 1H, H-9), 5.21 (s, 1H, H-1), 5.10−5.00 (m, 2H, H-4 and H-5), 4.15 (d, J = 8.1 Hz, 1H, H-6_{endo}), 4.05 (dd, J = 8.2 Hz, J = 5.3 Hz, 1H, H-6_{exo}), 3.24 (d, J = 17.4 Hz, 1H, H-3_{eq}), 3.06 (dd, J = 17.3 Hz, J = 7.3 Hz, 1H, H-3_{ax}), 2.84 (s, 1H, —OH); ^{13}C NMR ($CDCl_3$) δ 195.8 (C, C-2), 151.5 (C, C-8), 141.4 (C, arom), 132.5 (CH, C-7), 128.6 (CH, arom), 128.1 (CH, arom), 126.3 (CH, arom), 101.3 (CH, C-1), 76.0 (CH, C-5), 69.2 (CH, C-9), 65.6 (CH_2, C-6), 63.1 (CH, C-4), 34.9 (CH_2, C-3); HRMS calcd for $C_{15}H_{15}N_3O_4Na$ (M + Na) 324.0955, found 324.0968.

Compound **7k**: 28.2 mg, 71% yield; yellowish glass; $[\alpha]_D^{28}$ −182.1 (*c* 1.00, $CHCl_3$); IR (film) ν_{max} 2958, 2920, 1748 (C═O), 1504, 1463, 1226, 1115, 1033, 968, 908, 881 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (s, 1H, H-7), 6.94−6.80 (m, 4H, arom), 5.23 (s, 1H, H-1), 5.09 (s, 2H, H-9), 5.14−5.04 (m, 2H, H-4 and H-5), 4.18 (dd, J = 8.3 Hz, J = 1.1 Hz, 1H, H-6_{endo}), 4.07 (dd, J = 8.1 Hz, J = 5.4 Hz, 1H, H-6_{exo}), 3.77 (s, 3H, H-10), 3.27 (ddd, J = 17.2 Hz, J = 3.3 Hz, J = 1.3 Hz, 1H, H-3_{eq}), 3.09 (dd, J = 17.3 Hz, J = 7.3 Hz, 1H, H-3_{ax}); ^{13}C NMR ($CDCl_3$) δ 195.7 (C, C-2), 154.2 (C, arom), 152.2 (C, arom), 145.0 (C, C-8), 134.1 (CH, C-7), 115.8 (CH, arom), 114.5 (CH, arom), 101.3 (CH, C-1), 76.0 (CH, C-5), 65.6 (CH_2, C-6), 63.2 (CH, C-4), 62.2 (CH_2, C-9), 55.6 (CH_3, C-10), 34.9 (CH_2, C-3); HRMS calcd for $C_{16}H_{17}N_3O_5Na$ (M + Na) 354.1060, found 354.1068.

Procedure for the ^1H NMR Kinetics Experiments. *Isomerization of* **2**. The crude material containing **2** (approximately 0.28 mmol) was transferred to an NMR tube and dissolved in $CDCl_3$ (0.8 mL). After the first ^1H NMR spectrum was taken, 15 μL of a triethylamine solution (2.8 M in $CDCl_3$) was quickly added and the second spectra was taken at room temperature. A series of spectra were taken at regular intervals of time (40−60 min) until significant conversion toward **6** was noted. With the different chemical shifts in the ^1H NMR signals exhibited by the starting material, the corresponding isomerized product and levoglucosenone allowed the determination of the progress of the reaction by integration of those signals in each ^1H NMR spectrum. With the integral values, we calculated the proportion of these three components over time.

Isomerization of **4a**. **4a** (0.12 mmol) was transferred to an NMR tube and dissolved in $CDCl_3$ (0.6 mL). After the first ^1H NMR spectrum was taken, 100 μL of a triethylamine solution (1.2 M in $CDCl_3$) was quickly added and the second spectrum was taken at room temperature. Afterward, the NMR tube was immediately placed in a silicone oil bath at 70 °C. A series of spectra were taken at regular intervals of time (40−60 min) until significant conversion toward **7a** was noted. With the different chemical shifts in the ^1H NMR signals exhibited by the starting material, the corresponding isomerized product and levoglucosenone allowed the determination of the progress of the reaction by integration of those signals in each ^1H NMR spectrum. With the integral values, we calculated the proportion of these three components over time.

Cell Culture and Survival Assay. The human breast adenocarcinoma cell line MDA-MB-231 was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum, penicillin G (100 units/ml, Sigma), and streptomycin (100 μg/mL, Sigma) and maintained in a 5% CO_2 humidified incubator at 37 °C. Cells with stable expression of short hairpin RNAs (shRNAs) targeting p53 or control were generated by transduction with retroviral particles containing plasmids pSRshp53 (5′-GACUCCAGUGGUAAUCUAC-3′) or pSRshLacZ as a control (5′- GUGACCAGCGAAUACCUGU-3′) and were selected with puromycin.[24] P53 knockdown was confirmed by Western blot using p53 antibody (DO-1 Santa Cruz) and anti-actin (A2066, Sigma) as a loading control. Cell viability was analyzed using the MTT assay. Briefly, 24 h prior to treatment, cells were seeded in 96-well plates at a density of 7 × 103 cells per well. The synthesized compounds were dissolved in DMSO at 20 mM and then diluted in culture medium to achieve the final concentration for the different treatments. As negative controls, cells were incubated with the corresponding concentration of DMSO (Merck), according to the dilution. After 48 h of treatment, cells were stained with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (0.5 mg/mL; Sigma) for 4 h at 37 °C. After the removal of the culture medium, formazan crystals were dissolved in DMSO and the absorbance was measured at 540 nm using a microplate reader.

Computational Methods. All of the quantum mechanical calculations were performed using Gaussian 09.[25] The conformational search was done in the gas phase using the MMFF force field (implemented in Spartan 08).[26] All conformers found were subjected to further reoptimization at the B3LYP/6-31G* level of theory. The conformations within 2 kcal/mol from the B3LYP/6-31G* global minima were subjected to NMR calculations. The magnetic shielding constants (σ) were computed using the gauge including the atomic orbitals (GIAO) method,[27] the method of choice to solve the gauge origin problem,[18] at the PCM/mPW1PW91/6-31+G** levels of theory. The calculations in solution were carried out using the polarizable continuum model, PCM,[28] with chloroform as the solvent. The unscaled chemical shifts (δ_u) were computed using TMS as a reference standard according to $\delta_u = \sigma_0 - \sigma_x$, where σ_x is the Boltzmann averaged shielding tensor (over all significantly populated conformations) and σ_0 is the shielding tensor of TMS computed at the same level of theory employed for σ_x. The Boltzmann averaging was done according to eq 1:

$$\sigma^x = \frac{\sum_i \sigma_i^x e^{(-E_i/RT)}}{\sum_i e^{(-E_i/RT)}} \quad (1)$$

where σ_i^x is the shielding constant for nucleus x in conformer i, R is the molar gas constant (8.3145 J K^{-1} mol^{-1}), T is the temperature (298 K), and E_i is the energy of conformer i (relative to the lowest energy conformer), obtained from the single-point NMR calculation at the corresponding level of theory. The scaled chemical shifts (δ_s) were computed as $\delta_s = (\delta_u - b)/m$, where m and b are the slope and intercept, respectively, resulting from a linear regression calculation on a plot of δ_u against δ_{exp}. The DP4+ calculations were carried out using the Excel spreadsheet available for free at sarotti-NMR.weebly.com or as part of the Supporting Information of the original paper.[20]

The transition structures (**TS-2**, **TS-6**, **TS-4a**, and **TS-7a**) and the corresponding adducts (**2**, **6**, **4a**, and **7a**) were fully optimized at the M06-2*X*/6-31G* level of theory. The reported thermochemical properties include zero-point energies (ZPEs) without scaling and were calculated at 1 atm and 343 K. Normal mode analysis was used to confirm the nature of the stationary points and to evaluate the thermochemical properties. All transition structures were confirmed to have only one imaginary frequency corresponding to the formation of the expected bonds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b03141.

Additional biological essays, B3LYP/6-31G* and M06-2X/6-31G* optimized geometries, GIAO isotropic shielding tensors, detailed DP4+ probabilities and Cartesian coordinates of compounds **2**, **6**, **4a**, **5a**, **7a**, and **8a**, single-crystal X-ray diffraction experiments, and copies of the ^1H and ^{13}C NMR spectra of all compounds (PDF)

Crystal data for **7a-OH** (CIF)

Crystal data for **5a** (CIF)

Crystal data for **4a** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sarotti@iquir-conicet.gov.ar.

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ORCID

Felipe Terra Martins: 0000-0001-9004-0927
Rolando A. Spanevello: 0000-0003-3701-5807
Ariel M. Sarotti: 0000-0002-8151-0306

Notes

The authors declare no competing financial interest.

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