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Chromophore-Supported Purification in Parallel Synthesis

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The requirement for chromatographic separation and purification of product mixtures of greater or lesser degrees of complexity slows down every synthesis and is especially unfavorable when a series of parallel reactions is carried out in order to produce a library of structurally diverse compounds. To speed up chromatography, we have employed guajazulene derivatives to "dye" the starting material of a given reaction. The blue color of the chromophore-marked reaction products facilitates visual inspection of the separation process during column chromatography, allowing many columns to be carried out in parallel. In addition to their function as color markers, the employed blue guajazulene derivatives can also be used as protecting groups during the synthesis. We have named this methodology "chromophore-supported purification" (CSP) and have demonstrated its value in two parallel syntheses: in parallel acylations of the 6-position of a chromophore-marked mannoside on the one hand, and in the employment of CSP for workup after parallel 1,3-dipolar cycloadditions between guajazulene-marked alkynes and sugar azides on the other.

Introduction

In the era of combinatorial chemistry, parallel synthesis has become an important methodology in drug research. It is often used for the synthesis of small, focused libraries, in which the structural diversity of a given ligand, agonist or antagonist, is systematically varied to optimize its biochemical properties.[1] Time is a critical parameter for an effective parallel synthesis, in which each compound is prepared in an individual well and has to be purified individually. The parallel execution of reactions is thus not the only requirement for rapid production of compound libraries, workup procedures also have to be simplified to be less time-consuming. This has stimulated the search for alternative strategies for workup and purification,[2] including fluorous tagging[3] and the use of solid supports.[4] Solid-phase synthesis has solved some of the separation problems, but is associated with a number of other problems, most notably the need for suitable linker units and limitation to certain reactions and reaction conditions. Separation of side products remains a notorious problem in solid-phase synthesis, which therefore depends on careful optimization of reaction conditions and yields. Once the analysis of a product mixture is intended, and especially when this requires chromatographic steps, the separation and purification of products and byproducts can become the bottleneck of a synthesis.

The development of carbohydrate ligands for carbohydrate-binding proteins is typically affected by the described problems, and so we sought for a method to facilitate workup of carbohydrates and to speed up the chromatographic separation and purification of a product mixture without any requirement for optimized reaction conditions. We decided to tag the starting material with a suitable color marker molecule to allow a "chromophore-supported purification" ("CSP"). As chromatographic purification of a product mixture is essential for many reactions and compound classes, especially in the areas of natural products and bioactive compounds, CSP should be of broad advantage in organic chemistry, once an appropriate color label is found.

Results and Discussion

We selected azulenes as our color tags for CSP as they are stable enough to be introduced into a large number of starting materials and to survive a variety of reaction conditions. Furthermore, their derivatives are well suited for adsorption chromatography, thanks to their good lipophilicity. We started with guajazulene (**1**), an inexpensive azulene derivative, which was converted into the carboxylic acid **3** by treatment with bromoacetic acid as described in the literature[5] and into the alcohol **4** by treatment with paraformaldehyde (Scheme 1). Both reactions start with the deprotonation of **1**, to produce the resonance-stabilized guajazulene anion **2**.

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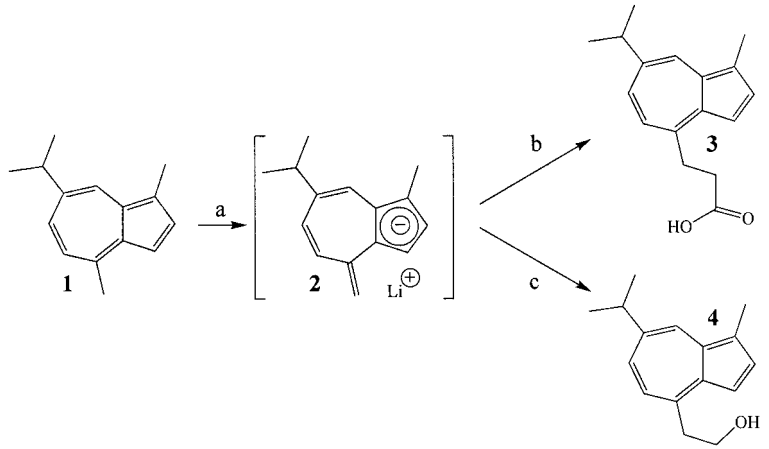
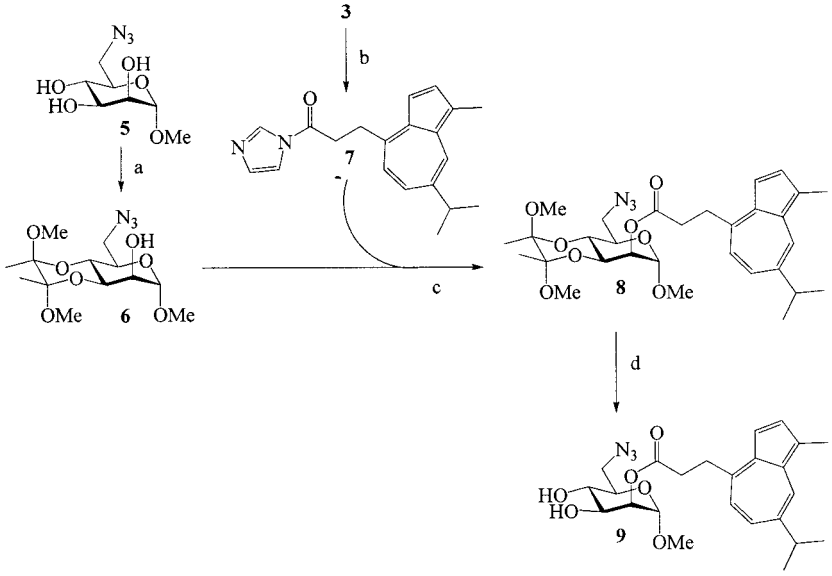


Scheme 1. a) LDA, diethyl ether, –35 °C, 40 min. b) BrAcOH, –35 °C (10 min), then 2 h room temp., 37% (61% based on conversion). c) $(CH_2O)_n$, –35 °C (10 min), then room temp. overnight, 32% (46% based on conversion).

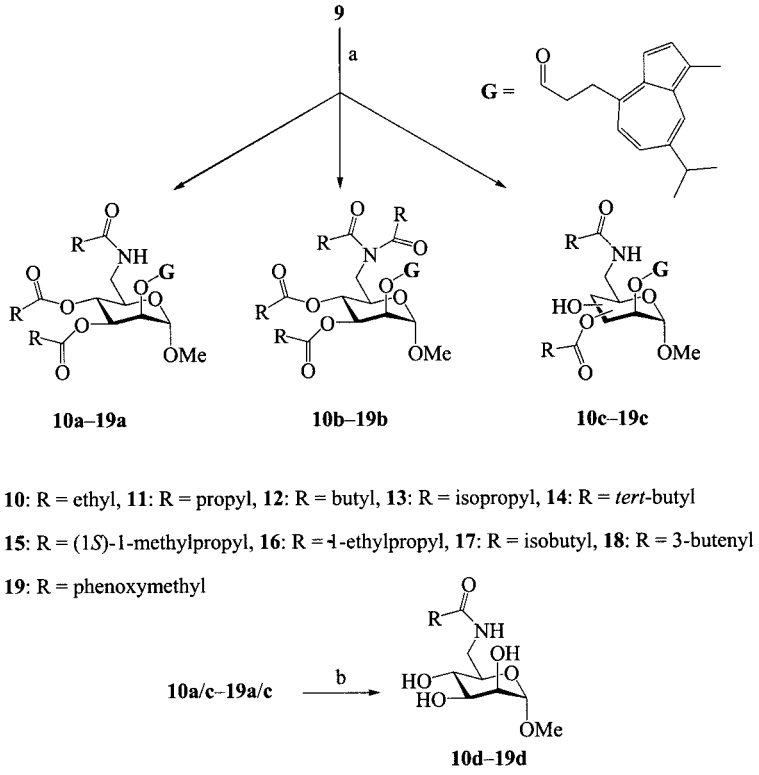
The guajazulene derivatives **3** and **4** are both deep blue in color, which is typical for azulenes and qualifies both compounds as markers for CSP. Their potential was evaluated in the context of two parallel synthetic sequences, both aimed at the structural variation of mannose ligands to increase their affinity for mannose-specific lectins.[6]

The first synthetic sequence started with the 6-azido-6-deoxymannoside **5**,[7] which can be obtained from mannose in three steps. The terminal azido function is needed for the regioselective modification of the 6-position of the sugar ring with so-called "enhancer moieties" in order to investigate and improve interaction with carbohydrate-binding proteins.[8] Treatment with diacetyl in methanol gave the 3,4-*O*-protected mannoside **6**, ready for the introduction of the guajazulene chromophore in the 2-position. The guajazulene derivative **3** was first converted into its imidazolide **7** with the aid of 1,1′-carbonyldiimidazole (CDI) in DMF (Scheme 2), and this was then treated with the alcohol **6** in a DBU-catalyzed reaction to deliver the blue ester **8**. Thiolysis of the diacetal protecting group with dimercaptopropane (DMP) gave the azido-functionalized diol **9**, the substrate for the subsequent parallel syntheses, in which ten commercially available carboxylic acid anhydrides carrying alkyl, alkenyl, or aryl residues (Scheme 3) were used to modify the 6-position of the α-mannoside. Trimethylphosphane was employed in these reactions, causing activation both of the OH groups in the 3- and 4-positions and of the azide group in the 6-position of the sugar ring. Accordingly, these reactions afforded mixtures of products, consisting of the di-*O*-acylated *N*-acylamides **10a**–**19a**, the corresponding di-*O*-acylated *N*,*N*-diacylamides **10b**–**19b**, and the mono-*O*-acylated *N*-acylamides **10c**–**19c** (Scheme 3).

For the evaluation of the CSP methodology with this small library of mannosides it was less important to optimize the Staudinger-type reaction with **9** than it was to determine whether CSP would allow rapid parallel purifica-



Scheme 2. a) 1. Butane-2,3-dione, $HC(OMe)_3$, BF_3 etherate, MeOH, room temp., 20 h. b) CDI, DMF, room temp., 1 h. c) DBU, DMF, 0 °C, overnight, 46% starting with **5**. d) DMP, $H[BF_4]$, dichloromethane, room temp., 2 h, 78%.



Scheme 3. a) $P(Me)_3$, $(RCO)_2O$, THF, room temp., overnight. b) NaOMe, MeOH, room temp., 1 h.

tion of the product mixtures without further optimization attempts. Thus, after the acylation reactions with **9** were complete, the solvent was removed in vacuo and the obtained product mixtures were subjected to parallel column chromatography. During column chromatography the separation of products and byproducts could be followed by visible inspection, and collection of the colored fractions was easily accomplished with ten columns running in parallel. The collected fractions were examined by MALDI-TOF mass spectrometry, revealing an additional advantage of the guajazulene label: all guajazulene-conjugated compounds were amenable to laser desorption without an additional matrix, giving very clean mass spectra reflecting only the molecular peaks.

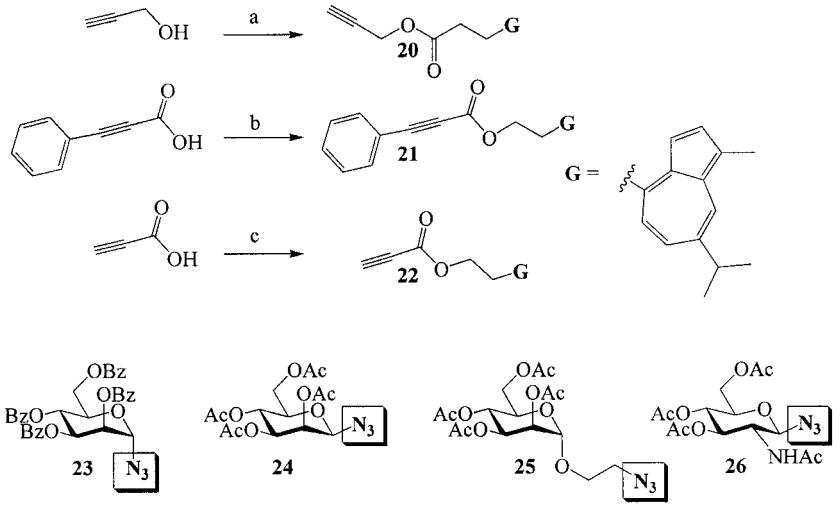
The results of mass spectrometric analysis of the reaction products are summarized in Table 1. Naturally, the regiochemical outcome of the reaction affording the differently *O*-acylated products **10c**–**19c**, was not determined by mass spectrometry and is of minor interest with regard to the eventual deacylation step to provided the deprotected products of the **d**-series. Table 1 shows that in most cases the triacylated mannosides of the **a**-series (**10a**–**19a**) were the predominantly formed products of the acylation reaction with the mannoside **9**, whilst the *N*,*N*-diacylated derivative (**b**-series; Scheme 3) never constituted the major product.

O-Deprotection of the products of the **a**- and **c**-series as described by Zemplén[9] also removed the chromophore in the 2-position to yield the unprotected amides **10d**–**19d**, which could be unequivocally characterized by NMR spectroscopy (see Supporting Information).

In addition to the conversion of the chromophore-marked mannoside **9**, we have also tested the value of CSP

Table 1. Results of the parallel acylations of mannoside **9** with ten different carboxylic acid anhydrides in the acylation entries **10** to **19** (cf. Scheme 3). *O*-Deprotection of the products of the **a**- and **c**-series gave amides of type **d** (**10d** to **19d**).

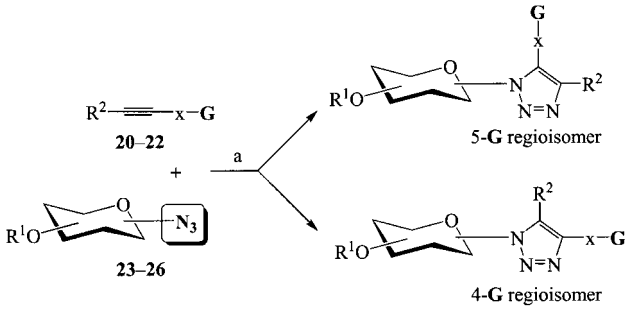
Acylation entry	Major product (% yield)	$M_{calcd.}$	Side product (% yield)	Deprotected product (**d**-series), % yield	MALDI-TOF-MS $[M + Na]^+$	MALDI-TOF-MS $[M + K]^+$	$M_{cald.}$
10	**a** (60)	599.31	–	95	272.1	288.1	249.12
11	**a** (70)	641.36	**b** (12)	94	286.0	302.0	263.14
12	**a** (61)	683.40	–	85	300.1	316.0	277.15
13	**a** (54)	641.36	**b** (13)	80	286.1	302.1	263.14
14	2× **c** (47)	599.35	**a** (10)	88	300.1	316.0	277.15
15	**a** (49)	683.40	**c** (27)	79	300.1	316.0	277.15
16	**c** (54)	627.38	**a** (20)	88	314.1	330.0	291.17
17	**a** (77)	683.40	**b** (8)	78	300.1	316.0	277.15
18	**a** (46)	677.36	**c** (21)	97	298.1	314.0	275.14
19	**a** (53)	833.34	–	quant.	350.0	365.9	327.13



Scheme 4. a) **7** (freshly prepared), DBU, 5 h, 94%. b) 1. DCC, diethyl ether, 0 °C, 0.5 h, 2. **4**, TEA, 0 °C, 71%. c) **4**, DCC, DMAP, THF, –20 °C (4 h), then room temp., overnight, 47%.

in a series of parallel reactions in which 1,3-dipolar cycloadditions between azides and alkynes have been employed for the introduction of molecular diversity in carbohydrate derivatives.[10] The alkyne component was chromophore-labeled either with the carboxylic acid derivative **3** or with the guajazulene alcohol **4**. Thus, the propargylic ester **20** was obtained with **3** in a CDI-mediated reaction, as described for the synthesis of **8**, while the alcohol **4** was used together with DCC as the coupling reagent for the preparation of the color-labeled alkynes **21** und **22** (Scheme 4). As azide components for the cycloaddition reaction the mannose derivatives **23**,[11] **24**,[11] and **25**,[12] and the GlcNAc derivative **26**[13] were selected and prepared by known literature procedures.

The guajazulene-protected alkynes **20**, **21**, and **22** were combined with the sugar azides **23**–**26** in 12 parallel reactions, to yield two regioisomeric triazoles in each case; these were named 4-**G** and 5-**G**, corresponding to the position of the guajazulene moiety in the five-membered triazole ring (Scheme 5). Again, our goal was not to improve the regioselectivity of this reaction,[14] but we intended rather to test whether CSP would allow the separation of the regioisomeric products of this cycloaddition reaction.



Scheme 5. 1,3-Dipolar cycloadditions between sugar azides and guajazulene-marked alkynes. a) 70 °C, 3–10 d. **G** = guajazulene chromophore; *x* = molecular moiety linking the chromophore to the alkyne (cf. Scheme 4).

Out of the 24 theoretically possible products of the 12 parallel syntheses carried out, 22 were obtained after several days' reaction time. The azide **23** and alkyne **21** did not undergo the expected reaction.

The parallel chromophore-supported separation of 11 regioisomeric mixtures thus became the key step of this series of reactions. In eight cases, separation of the regioisomeric products was visible on the column and fractioning of the products was easy. Cycloaddition products arising from the

Table 2. Twelve parallel 1,3-dipolar cycloadditions were carried out with the carbohydrate azides **23**, **24**, **25**, and **26** and the guajazulene-marked alkynes **20**, **21**, and **22** (cf. Scheme 4 and Scheme 5), forming two regioisomeric products in each reaction (the protected 4-**G** and the 5-**G** isomers, listed with their isolated yields after chromatography). If not otherwise indicated, the purities of the products were assumed to be >95% on the basis of their clean ^{1}H NMR spectra.

	23	**24**	**25**	**26**
20	4-G isomer: 63%	4-G isomer: 44%	4-G isomer: 40%	4-G isomer: 60%
	5-G isomer: 8%	5-G isomer: 32%[a]	5-G isomer: 25%[b]	5-G isomer: 31%[c]
21	4-G isomer: –	4-G isomer: 9%[d]	4-G isomer: 45%	4-G isomer: 41%
	5-G isomer: –	5-G isomer: 10%	5-G isomer: 41%	5-G isomer: 19%
22	4-G isomer: 78%	4-G isomer: 73%	4-G isomer: 81%	4-G isomer: 67%
	5-G isomer: 5%	5-G isomer: 14%[e]	5-G isomer: 17%	5-G isomer: 1%

[a] Purity according to the ^{1}H NMR spectrum: 83%. [b] Purity according to the ^{1}H NMR spectrum: 87%. [c] Purity according to the ^{1}H NMR spectrum: 91%. [d] Purity according to the ^{1}H NMR spectrum: 92%. [e] Purity according to the ^{1}H NMR spectrum: 88%.

combinations **20/24**, **20/25**, and **22/26** had to be separated by repeated column chromatography. Table 2 summarizes the yields of the 12 parallel cycloaddition reactions carried out. The purities of the separated products were assumed to be greater than 95% from their clean ^{1}H NMR spectra. Twenty triazoles were thus obtained in significant yields and subjected to deprotection under Zemplén conditions,[9] which removed the sugar protecting groups together with the guajazulene chromophore. The structures of the deprotected triazoles were unequivocally assigned by MALDI-TOF mass spectrometry and NMR spectroscopy, in particular by NOESY und HMBC NMR experiments (see Supporting Information).

Conclusions

In conclusion, it has been shown that a methodology in which reaction partners are marked with a suitable chromophore moiety allows parallel separation and purification of products and byproducts during column chromatography. Column chromatography of colored products is greatly facilitated in comparison to the separation of a colorless product mixture as the separation can be visually inspected. This chromophore-supported purification (CSP) was shown to be efficient in the acylation of mannoside **9**, in which ten columns were run in parallel, allowing the isolation of ten major products and seven side products in less than one day. In a second example, CSP was successfully employed for the parallel purification of 11 reaction mixtures, allowing the separation of 22 chemically very similar regioisomeric products of 1,3-dipolar cycloaddition reactions during a single day.

CSP requires an additional synthetic step for the introduction of the color marker, but the chromophores used for CSP can also be regarded and utilized as protecting groups and thus offer an additional advantage. CSP requires no challenging know-how nor sophisticated equipment and, in contrast to solid-phase synthesis, is compatible with reactions that produce mixtures of regioisomers or diastereomers. CSP facilitates reaction control and allows in-process optimization of the synthesis. With CSP a parallel synthesis approach might also become reasonable for a number of reactions previously regarded as not suited for parallel synthesis. CSP should be a feasible method to support purification of focused libraries of up to 100 compounds. In addition, this method can be employed on a scale of up to several grams, which is rather unusual for solid-phase chemistry.

Experimental Section

3-(7-Isopropyl-1-methylazulen-4-yl)propanoic Acid (3): A solution of LDA (12.6 mL, 25.2 mmol) in cyclohexane/ethylbenzene/THF was diluted with diethyl ether (60 mL) and cooled to –35 °C, and a solution of guajazulene (5.00 g, 25.2 mmol) in dry diethyl ether (15 mL) was then added dropwise under argon. The reaction mixture was stirred for 40 min and bromoacetic acid (1.75 g, 12.6 mmol) was then added at –35 °C. After 10 min the mixture was allowed to attain room temp., satd. aq. NaCl solution (300 mL) and diethyl ether (300 mL) were added after 2 h, the phases were separated, the organic phase was extracted with satd. aq. NaCl solution (100 mL), and the combined aqueous phases were washed with diethyl ether (3×, 100 mL). Diethyl ether was then carefully layered over the aqueous phase and aq. hydrochloric acid (2 N) was added until the blue product had been completely transported into the organic phase. The organic phase was then washed neutral and dried (Na_2SO_4), and the solvent was removed in vacuo after filtration. Chromatographic purification (toluene/ethyl acetate, 3:1) afforded the title compound (2.40 g, 9.34 mmol, 37%), in the form of blue crystals. m.p. 138 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 8.14 (d, $J_{6,8}$ = 2.0 Hz, 1 H, 8-H), 7.66 (dq, $J_{2,3}$ = 3.8 Hz, 1 H, 2-H), 7.43 (dd, $J_{5,6}$ = 10.6 Hz, $J_{2,Me}$ = 0.6 Hz, 1 H, 6-H), 7.29 (d, 1 H, 3-H), 7.03 (d, 1 H, 5-H), 3.49 (m_c, 2 H, CH_2CH_2COOH), 3.08 [sept, 1 H, $(CH_3)_2CH$], 2.90 (m_c, 2 H, CH_2COOH), 2.67 (d, 3 H, aryl-CH_3), 1.36 [d, $J_{isopropyl}$ = 6.9 Hz, 6 H, $CH(CH_3)_2$] ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 179.4 (COOH), 146.0 (C-4), 140.5 (C-7), 136.8 (C-2), 136.5 (C-8a), 136.4 (C-3a), 135.3 (C-6), 133.6 (C-8), 125.6 (C-1), 124.2 (C-5), 112.1 (C-3), 38.3 [$CH(CH_3)_2$], 35.3 (CH_2COOH), 32.9 (CH_2CH_2COOH), 24.7 [$CH(CH_3)_2$], 12.9 (aryl-CH_3) ppm. IR (KBr): 3093–2507 (COOH), 2962, 2926, 2866 (CH_{aliph}), 1701 (C=O) cm^{-1}. HR-MS: *m*/*z* = 256.14630 (*m*/*z* = 256.14633 calcd. for $C_{17}H_{20}O_2$).

4-(2-Hydroxyethyl)-7-isopropyl-1-methylazulene (4): A solution of LDA (12.6 mL, 25.2 mmol) in cyclohexane/ethylbenzene/THF was diluted with dry diethyl ether (60 mL) and cooled to –35 °C. A solution of guajazulene (5.00 g, 25.2 mmol) in dry diethyl ether (15 mL) was added dropwise under argon. The mixture was stirred for 40 min, and paraformaldehyde (756 mg, 25.2 mmol formaldehyde) was then added. After 10 min the cooling was removed and the reaction mixture was stirred overnight at room temp. Diethyl ether (100 mL) was added and the system was washed three times with satd. aq. NaCl. The organic phase was dried (Na_2SO_4), filtered, and concentrated, and the residue was purified by flash column chromatography (toluene/ethyl acetate, 6:1) to yield the title compound (1.53 g, 7.74 mmol, 32%) as a blue oil. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ = 8.20 (d, $J_{6,8}$ = 2.1 Hz, 1 H, 8-H), 7.65 (dq ≈ dd, $J_{2,3}$ = 3.8 Hz, $J_{2,Me}$ = 0.6 Hz, 1 H, 2-H), 7.43 (dd, $J_{5,6}$ = 10.7 Hz, 1 H, 6-H), 7.30 (d, 1 H, H-3), 7.02 (d, 1 H, 5-H), 4.04 (t, $J_{a,b}$ = 6.5 Hz, 2 H, CH_2OH), 3.38 (t, 2 H, CH_2CH_2OH), 3.07 [sept, $J_{isopropyl}$ = 6.9 Hz, 1 H, $CH(CH_3)_2$], 2.67 (d, 3 H, aryl-CH_3), 1.36 [d, 6 H, $CH(CH_3)_2$] ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 144.3 (C-4), 140.2 (C-7), 137.6 (C-2), 136.7, 136.1 (C-3a, C-8a), 135.1 (C-6), 133.6 (C-8), 125.6 (C-1), 124.8 (C-5), 112.4 (C-3), 63.6 (CH_2O), 41.3 (CH_2CH_2O), 38.3 [$CH(CH_3)_2$], 24.7 [$CH(CH_3)_2$], 12.9 (aryl-CH_3) ppm. IR (film): 3346 (br. s, OH), 2958 (CH_{aliph}) cm^{-1}. HRMS: *m*/*z* = 228.15120 (*m*/*z* = 228.15141 calcd. for $C_{16}H_{20}O$). MALDI-TOF MS (no matrix required): *m*/*z* = 228.1 $[M]^+$ (228.15 calcd. for $C_{16}H_{20}O$).

Supporting Information (see also the footnote on the first page of this article): The experimental procedures for the synthesis of the guajazulene derivatives **20**–**22** and full NMR and MS analytical data for all synthesized unprotected amides (cf. Scheme 3) and glyco-triazoles (cf. Scheme 5) are provided.

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