Functionalized 1,2-Dioxetanes as Potentially Useful Biological and Chemotherapeutic Agents

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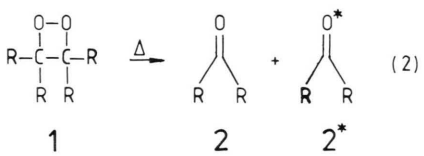
Hydroxymethyl-substituted 1,2-dioxetanes (**3**) have been converted in reasonable yields (10–40%) into the carboxylate-substituted 1,2-dioxetanes (**4**) by means of the Brewster–Ciotti or Mitsunobu esterification and into the tosylate-substituted 1,2-dioxetanes (**5**) by means of tosyl chloride in pyridine. The fact that hydroxy-functionalized 1,2-dioxetane can be chemically attached to carboxylic acids and sulfonic acids whit preservation of the dioxetane moiety opens up new opportunities for biomedical applications.

In photoaffinity labeling [1] a biochemically important target molecule (T), *e.g.* a substrate, hormone, allosteric effector, *etc.*, is modified by attaching a photolabile group. In an ideal situation the modified substrate is still reversibly bound to its receptor (R), *e.g.* an enzyme or specific protein. Subsequent irradiation generates a reactive species which now binds irreversibly to the receptor (eq. (1)). One can in this way obtain information on the location and structure of

$$T + R \rightleftarrows T\cdots R \xrightarrow{h\nu} T-R \qquad (1)$$

the receptor binding site. To the best of our knowledge, photoaffinity labeling has been conducted only *in vitro* and may be handicapped by the fact that the photo-generated reactive species is largely wasted by reaction with water.

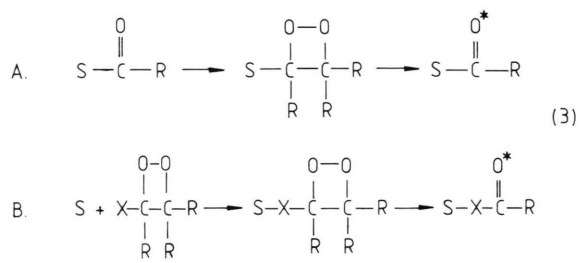
While mainly azides and diazoalkanes are utilized as photo-active labels [1], also electronically excited carbonyl groups qualify [2]. This special case of photoaffinity labeling provides us with the unique opportunity of exploiting 1,2-dioxetanes (**1**) for the purpose of "photoaffinity without light" [3]. As eq. (2)



(2)

1 **2** **2***

illustrates, on thermal decomposition, the 1,2-dioxetane (**1**) dissociates into two carbonyl products (**2**), of which one is electronically excited (**2***); usually the chemically reactive triplet n,π^* state is produced [4]. Therefore, in principle two strategies could be followed to perform dioxetane-energized photoaffinity labeling. Either the carbonyl group of the biological target molecule, *e.g.* a steroidal hormone, could be masked in form of a dioxetane (alternative A, eq. (3)) or a



(3)

dioxetane with a functional handle could be attached to the biological target molecule (alternative B, eq. (3)). Thermal activation under physiological conditions would release the electronically excited carbonyl group for photochemical attachment at the receptor site. In contrast to "optical" photoaffinity labeling, such "chemical" photoaffinity labeling could possibly be carried out *in vivo* because the usual problems of directing the radiation to the specific site are obviated. The potential of this unique strategy for biological testing and for developing new chemotherapeutic agents can hardly be overemphasized [3, 5].

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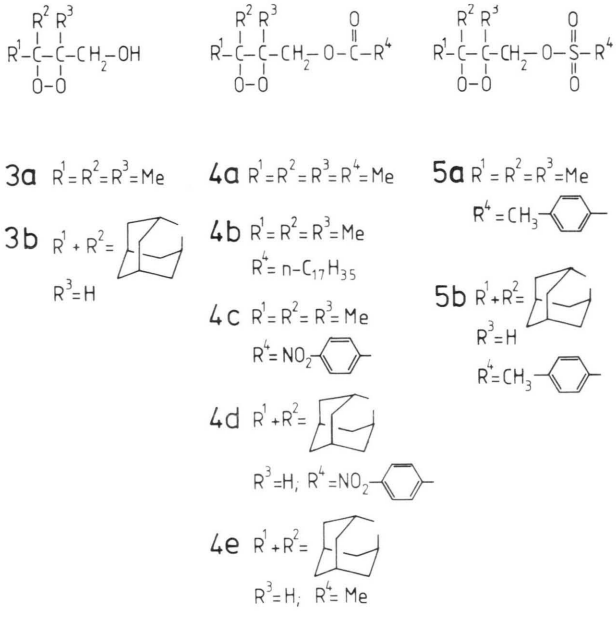
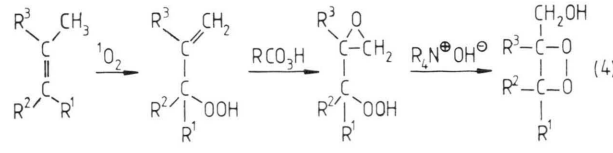
A fundamental requisite for the realization of the alternative B strategy is the availability of 1,2-dioxetanes with functional handles, which would permit attaching such latent *in situ* photoaffinity labels to the biological target molecule. In view of the labile nature of the dioxetanes, attachment of functionalized derivatives to the biological substrate demands sufficiently mild chemical transformations to assure preservation of the dioxetane moiety. We presently demonstrate the feasibility of such functional group manipulation of dioxetanes, with the conviction that these chemical model studies will pave the way for numerous biomedical applications.

The chemical transformation that we illustrate here is the esterification of hydroxymethyl-substituted dioxetanes (**3**) with carboxylic acids and sulfonic acids to result in the carboxylates (**4**) and the sulfonates (**5**), respectively. The hydroxymethyl-substituted dioxetanes (**3**) were prepared according to the synthetic sequence outlined in eq. (4) [6]. The spiroadamantane substitution in the dioxetane (**3b**) was chosen to provide additional chemical inertness [7] and thermal stability [8], should esterification lead to destruction of the dioxetane. For the conversion (**3**)→(**4**) the Brewster–Ciotti [9] and the Mitsunobu [10] esterification methods were used, while the conversion (**3**)→(**5**) employed tosyl chloride in pyridine [11]. Typical esterification procedures are given below and the yields, physical constants and spectral data are summarized in the Table.





As the results in the table show, the pure carboxylate- and tosylate-substituted dioxetanes (**4**) and (**5**), respectively, could be obtained in reasonable yields. In the case of the dioxetanes (**4**), the Mitsunobu esterification gave usually higher yields. In the Brewster–Ciotti esterification mixtures of carboxylate (**4**) and tosylate (**5**) were obtained. In fact, in the case of the sluggishly reacting stearic acid, the corresponding tosylate (**5**) was formed as major product. Nevertheless, the fact that hydroxymethyl-functionalized dioxetanes can be chemically linked to carboxylic acids with preservation of the dioxetane moiety, especially fatty acids such as stearic acid, opens up promising and challenging opportunities in biomedical research [3].

Experimental

Preparation of Carboxylates (**4**)

Brewster–Ciotti esterification [9]: A 25 ml Erlenmeyer flask, capped with a rubber septum, was provided with spinbar and charged with 12 mmol carboxylic acid and 24 mmol tosyl chloride in 25 ml anhydrous, freshly distilled pyridine, cooled to 0 °C, and while magnetically stirring was added by means of a syringe a precooled solution of 6 mmol hydroxymethyl-1,2-dioxetane (**3**) in 5 ml pyridine. Reaction progress was monitored by TLC on silica gel eluting with dichloromethane. After optimal yields of esterified dioxetanes (**4**) were achieved (usually 60 min at 13 °C), the reaction mixture was poured on *ca.* 50 g crushed ice, the mixture transferred to a separatory funnel and extracted with 3×20 ml methyl *t*-butyl ether. The combined extracts were washed with 3×10 ml 2 N-aqu. HCl and 10 ml water, dried over anhydr. MgSO4, the solvent rotoevaporated at 0 °C and 18 Torr and the peroxidic residue chromatographed on activity grade III silica gel (adsorbant to substrate ratio *ca.* 60:1) at −27 °C, eluting with dichloromethane. Solid dioxetane products were recrystallized for final purification. Purity was established by iodometry (KI in HOAc).

Mitsunobu esterification [10]: A 100 ml three-necked, round-bottomed flask, provided with spin-

Table. Yields, physical constants and spectral data of the carboxylate and tosylate-functionalized 1,2-dioxetanes (**4**) and (**5**).

Dioxetane	Method[a]	Yield [%][b]	Purity [%][c]	Phys. Const. m.p. [°C]	IR (CCl_4) [ppm]	^{1}H NMR (90 MHz; $CDCl_3$) [ppm]	^{13}C NMR (100 MHz; $CDCl_3$) [ppm]
4a	BC	16	97	yellow oil[d]	2940, 1755, 1425, 1375, 1250, 1230, 1190, 1040, 865, 690	1.40 (s; 3H, CH_3), 1.45 (s; 3H, CH_3), 1.65 (s; 3H, CH_3), 2.05 (s; 3H, CH_3), AB-pattern (δ_A=4.55, δ_B=4.28, J=11.7 Hz; 2H, CH_2)	17.89 (q), 20.84 (q, CH_3C=O), 22.13 (q), 23.92 (q), 66.08 (t, CH_2), 88.86 (s), 89.07 (s), 170.76 (s; C=O)
4b[e]	BC M	3 17	97 98	66–67 (pale yellow prisms)	2915, 2825, 1750, 1420, 1410, 1375, 1230, 1215, 1190, 1150	0.85 (t, J=6.72 Hz; 3H, $CH_2\underline{CH_3}$), 1.18–1.31 (m; 28H, $(\underline{CH_2})_{14}CH_3$), 1.42 (s; 3H, CH_3), 1.43 (s; 3H, CH_3), 1.60 (m; $COCH_2\underline{CH_2}$), 1.66 (s; 3H, CH_3), 2.34 (t, J=7.57 Hz; 2H, $CO-CH_2$), AB-pattern (δ_A=4.57, δ_B=4.51, J=11.49 Hz; 2H, OCH_2)	14.13 (q, $CH_2\underline{CH_3}$), 17.94 (q), 22.16 (q), 22.65 (q), 23.89 (t), 24.77 (t), 29.02 (t), 29.18 (t), 29.38 (t), 29.64 (overlapping CH_2's), 31.86 (t), 34.02 (t), 65.78 (t, CH_2O), 88.86 (s), 89.04 (s), 173.43 (s; C=O)
4c	BC	39	98	124–126 (yellow needles)	2950, 1740, 1540, 1350, 1280, 1250, 1120, 1105, 865, 720	1.45 (s; 6H, CH_3), 1.67 (s; 3H, CH_3), AB-pattern (δ_A=4.83, δ_B=4.73, J=12.0 Hz; 2H, CH_2) AA′BB′-pattern (δ_A=8.38, δ_B=8.20, J=9.6 Hz; 4H, p-$NO_2-C_6H_4-$)	17.94 (q), 22.16 (q), 24.01 (q), 67.26 (t, CH_2), 88.94 (s), 89.16 (s), 123.56 (d, ortho), 130.76 (d, meta), 134.69 (s, alpha), 150.47 (s, para), 164.27 (s; C=O)
4d	BC M	13 36	97 97	79 (yellow needles)	2900, 2840, 1740, 1535, 1355, 1275, 1130, 1105, 1020, 720	1.40–2.80 (m; 14H, adamantane), 4.93 (d, J=5.1 Hz; 2H, CH_2), 5.20 (t, J=5.1 Hz; 1H, CH), 8.15–8.45 (m; 4H, p-$NO_2-C_6H_4-$)	25.87, 25.99, 30.57, 31.45, 32.61, 33.36, 35.12, 35.73, 37.34, 64.18 (t; CH_2O), 86.87 (d; CH-dioxetane), 91.32 (s; spiro-dioxetane), 123.63 (d, ortho), 130.85 (d, meta), 134.65 (s, alpha), 150.56 (s, para), 164.36 (s; C=O)
4e[e]	BC	23	98	73–75 (yellow needles)	2920, 2860, 1755, 1570, 1550, 1460, 1375, 1240, 1045, 1010, 985	1.51–2.14 (m; 12H, adamantane), 2.09 (s; 3H, CH_3), 2.52 (m; 1H, adamantane), 2.70 (m; 1H, adamantane), ABX-pattern (δ_A=4.58, δ_B=4.62, J_{AX}=3.78 Hz; J_{BX}=4.04 Hz, J_{AB}=12.08 Hz; 3H, OCH_2CH)	20.83 (q; CH_3), 25.74, 25.92, 30.52, 31.38, 32.41, 33.17, 34.93, 35.68, 37.29, 62.80 (t; CH_2O), 86.98 (d; CH-dioxetane), 91.22 (s; spiro-dioxetane), 170.79 (s; C=O)
5a	T	30	99	55–56 (yellow needles)	3060, 3040, 2980, 1605, 1390, 1205, 1190, 1110, 1005, 670	1.28 (s; 3H, CH_3), 1.32 (s; 3H, CH_3), 1.60 (s; 3H, CH_3), 2.45 (s; 3H, CH_3), AB-pattern (δ_A=4.64, δ_B=4.03, J=9.5 Hz; 2H, CH_2), AA′BB′-pattern (δ_A=7.74, δ_B=7.32; J=9.6 Hz; 4H, MeC_6H_4-)	17.98 (q), 21.70 (q, $Ar-CH_3$), 22.11 (q), 23.86 (q), 70.60 (t; CH_2), 88.37 (s), 89.23 (s), 127.98 (d, ortho), 129.99 (d, meta), 131.53 (s, para), 145.40 (s, alpha)
5b	T	30	99	94–95 (yellow needles)	2900, 2850, 1455, 1380, 1210, 1195, 1180, 1100, 1050, 1005, 660	1.2–2.3 (m; 12H, adamantane), 2.45 (s; 3H, $-C_6H_4-CH_3$), 2.6–2.8 (m; 2H, adamantane), d of AB-pattern (δ_A=4.59, δ_B=4.45, J_{AB}=10.2 Hz, J=5.1 and 6.6 Hz; 2H, CH_2), 4.95 (dd, J=6.6 and 5.1 Hz; 1H, CH), AA′BB′-pattern (δ_A=7.84, δ_B=7.41, J=8.1; 4H, $Me-C_6H_4-$)	21.73 (q; CH_3), 25.75, 25.97, 30.45, 31.45, 32.42, 33.14, 34.73, 35.70, 37.09, 67.34 (t; CH_2CO), 86.53 (CH-dioxetane), 91.39 (s, spiro-dioxetane), 128.02 (d, meta), 130.06 (d, ortho), 131.94 (s, para), 145.42 (s, alpha)

[a] BC = Brewster–Ciotti esterification, Ref. [9]; M = Mitsunobu esterification, Ref. [10]; T = Tosylation, Ref. [11]; [b] isolated, purified yields; [c] by iodometry (KI in HOAc); [d] after silica gel chromatography at −27 °C, eluting with dichloromethane; [e] ^{1}H NMR measured at 400 MHz.

bar and three-way stopcock, was charged with 1 mmol of triphenylphosphin. After flame-drying under nitrogen atmosphere, 10 ml of diethyl ether (freshly distilled from $LiAlH_4$) and 1 mmol of freshly distilled ethyl azodicarboxylate were added, stirred for 5 min and at 0 °C 1 mmol of the carboxylic acid in 15 ml ether and 1 mmol hydroxymethyl-1,2-dioxetane (**3**) in 10 ml ether were syringed into the flask in that order. Reaction progress was monitored by TLC on silica gel eluting with dichloromethane. After optimal yields of esterified dioxetanes (**4**) were achieved (usually 2 h at 0 °C, but for the stearate (**4b**) 12 h at 10 °C) the solvent was roto-evaporated at 5 °C and 18 Torr, the residue triturated with 20 ml ligroin, the combined triturates washed with 2×20 ml water and dried over anhydr. $MgSO_4$. Roto-evaporation of the solvent at 0 °C and 18 Torr and chromatography of the residue on activity grade III silica gel (adsorbant to substrate ratio of 40:1) at −20 °C eluting with dichloromethane afforded the dioxetane products (**4**). Solid dioxetanes were recrystallized for final purification. Purity was established by iodometry (KI in HOAc).

Preparation of Tosylates (**5**)

The same procedure as outlined for the Brewster–Ciotti esterification was used, except that the carboxylic acid was left out. Furthermore, the solid tosylates (**5**) were purified as described in the Brewster–Ciotti esterification.

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