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Synthesis of the first fluorinated cantharidin analogues

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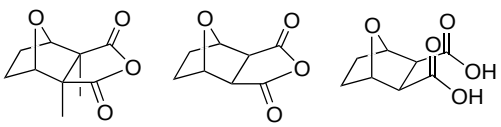
Abstract—Fluorinated cantharidin analogues (**4**–**7**) have been synthesized for the first time. The key step in each synthesis is an *exo*-selective Diels–Alder reaction of furan with the appropriate fluorinated maleic anhydride. ^{17}O NMR measurements of **5**–**7** and the corresponding non-fluorinated parent compounds were undertaken to show the influence of the fluorine substituent(s) on the electronic properties of the oxygen atoms.

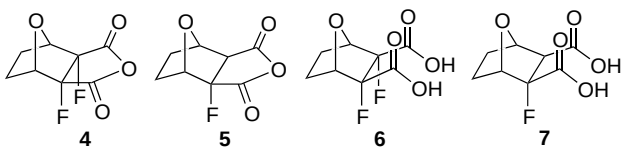
Cantharidin (**1**) is a naturally occurring toxin which has been isolated in the 'Spanish fly' *Cantharis vesicatoria*, and then found in many related *Mylabris* species.[1,2] Compound **1** has a rich history and a wide range of biological activities.[3] As a powerful vesicant it was used in the middle ages[1a] in Europe and as a traditional medicine in China over 2000 years ago[1b] to remove warts, although high toxicity of **1** limits general use.[4]



1 **2** **3**

This compound has been poisoning humans until now as a result of it's use as a supposed aphrodisiac ('Spanish fly') and it's abortificiant properties.[4] Cantharidin (**1**) has been used as an insecticide[3] and the hydrolysis product of norcantharidin (**2**), endothall (**3**), as a potent herbicide.[3,5] Furthermore, cantharidin (**1**) is the simplest and conformationally most rigid member of a group of serine/threonine protein phosphatase (PP) inhibitors. In the past few years, the effect of cantharidin (**1**) and its analogues as inhibitors of protein phosphatases, PP1, PP2a and PP2b, has been extensively investigated.[5–7] These phosphatases (PPs) play an important role in the regulation of many cellular processes. Generally, they act as cell proliferation inhibitors and/or tumor suppressors by opposing the actions of protein kinases that stimulate cell proliferation.[6] Several studies showed cantharidin (**1**) and its derivatives to possess potential anti-tumor capability[1b] against liver,[8] lung, colon and breast cancers.[9] Norcantharidin (**2**) is used as a routine anticancer agent already.[10] Improved inhibitor specificity is striven for with respect to both the organ and the type of protein phosphatase.[6]

Fluorine substitution often leads to modified biological activity and/or lowered toxicity compared to the corresponding non-fluorinated parent compounds.[11] Thus, fluorinated cantharidin analogues (e.g. **4**–**7**) represent a rewarding synthetic target, also with respect to an altered metabolism which was observed for α-monofluoroalkanoic acids.[12]



4 **5** **6** **7**

The key step in the synthesis of these compounds is a Diels–Alder reaction of furan with the appropriate fluorinated maleic anhydride. Several [4+2]-cycloadditions of fluorinated dienophiles are known.[13–15] Mostly *exo*-products[16] have been formed with cyclopentadiene or furan as the diene in the case of monofluorinated or *cis*-1,2-difluorinated vinylic compounds,[14] while *endo*-selectivity is mostly obtained in usual Diels–Alder reactions with non-fluorinated dienophiles.[17] Synthesis of the corresponding fluorinated *endo*-derivatives[16] seems not promising as, in the case of non-fluorinated derivatives, *endo*-compounds exhibited substantially decreased activity.[4]

Keywords: Diels–Alder reaction; fluorinated cantharidin analogues; fluorinated maleic anhydrides; furan; ^{17}O NMR.

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† X-Ray analysis.

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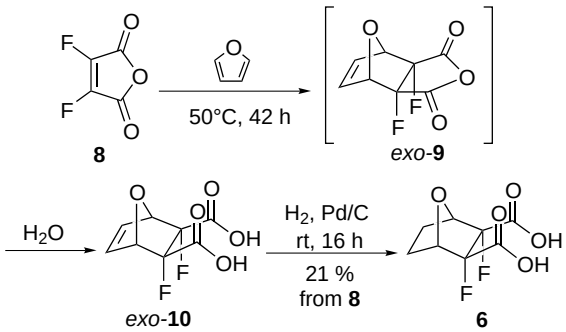
In this letter we report the straightforward synthesis of four fluorinated cantharidin analogues (**4**–**7**), which are additionally investigated by ^{17}O NMR measurements (compounds **5**–**7**) to gain information about the influence of the fluorine substituent(s) on the electronic properties of the oxygen atoms. It is likely that these oxygen atoms are responsible for binding to the active sites of PPs.[7d] Endothall (**3**) and some of its analogues form 1:1 complexes with Cu(II) whose stability is correlated to the toxicity of the endothall isomers.[18] Also, the crystal structure of a platinum(II)cantharidinate complex showed binding of the oxygen atoms towards metal centers.[9]



Scheme 1. Synthesis of 2,3-difluoroendothall **6**.

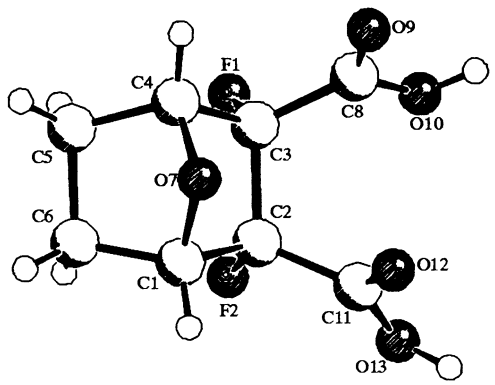
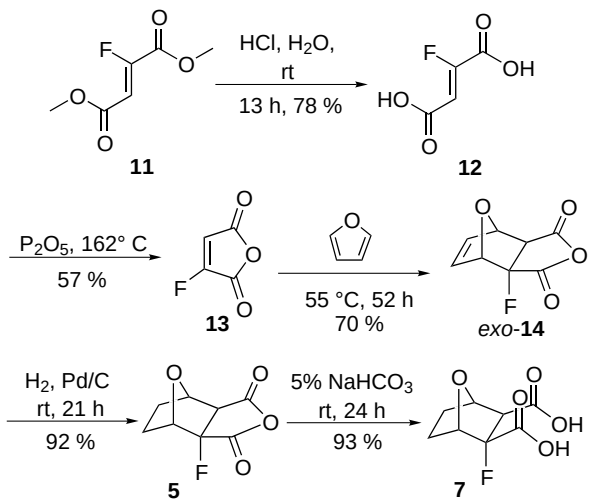


Figure 1. Crystal structure analysis of 2,3-difluoroendothall **6**.[22]



Scheme 2. Synthesis of 2-fluoronorcantharidin (**5**) and 2-fluoroendothall (**7**).

Syntheses. Our planned synthesis of **4** begins with the known difluoromaleic anhydride (**8**), which was obtained according to a literature procedure starting with oxidation of tetrafluoro-*p*-benzoquinone by peracetic acid to furnish difluoromaleic acid.[19] Difluoromaleic anhydride **8** was obtained by dehydration with P_2O_5.[20]

The latter reacted with furan in a Diels–Alder reaction to yield the *exo*-adduct[16] **10** stereoselectively (59%, crude). Surprisingly, the expected adduct *exo*-**9** was not observed, suggesting rapid hydrolysis of the latter on contact with moisture (Scheme 1). Attempts to facilitate the [4+2]-cycloaddition by microwave irradiation were not successful as only decomposition products have been observed. Compound **10**, on hydrogenation gave 2,3-difluoroendothall **6**[21] (21% with respect to **8**) whose X-ray analysis proves the *exo*-configuration (Fig. 1).

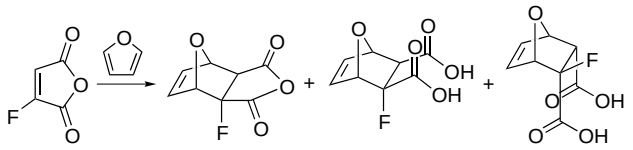
This synthesis is the first one to furnish the hitherto unknown **6** (in two steps starting from **8**). 2,3-Difluoronorcantharidin (**4**) was obtained as a 61:39 mixture of **6** and **4** (81%) after sublimation of **6** and handling under argon.

Synthesis of 2-fluoronorcantharidin (**5**) (Scheme 2) was accomplished in a four-step sequence starting from the known fluorofumaric acid dimethyl ester (**11**), which was obtained from commercially available dimethyl acetylene dicarboxylate according to Cousseau et al.[23] Compound **11** was hydrolyzed with aqueous HCl to furnish 78% of fluorofumaric acid (**12**). By this means, a new straightforward two-step route to **12** was found compared to the classical route by Castle et al. starting from chlorotrifluoro ethylene (four steps, 19% yield).[20]

Initial attempts to find an alternative short route to fluorofumaric acid (**12**) by bromofluorination[24] of but-2-ene-1,4-diol, subsequent oxidation to 2-bromo-3-fluorosuccinic acid, and its dehydrobromation to **12** suffered from low yield. Another sequence started with bromofluorination of *trans*-stilbene[14f,25] and subsequent RuO_4-oxidation of the bromofluoride to yield 2-bromo-3-fluorosuccinic acid. Again, yield and purity of the second step were unsatisfactory. Dehydration of **12** with P_2O_5[20] afforded fluoromaleic anhydride (**13**) in 57% yield.

This compound was stereoselectively converted under mild conditions (55°C) to Diels–Alder adduct *exo*-**14** with furan in a maximum yield of 70% (Table 1).[26] This [4+2]-cycloaddition can also be conducted in a domestic microwave oven with significantly shortened reaction time (1 h versus 52 h for the conventional thermal reaction), however with lower selectivity, due to partial hydrolysis of *exo*-**14**, and decreased yield (*exo*-**14**: 37%, *exo*-**15**: 29%, *endo*-**15**: 4%). 5,6-Didehydro-2-fluoroendothall (*exo*-**15**) was selectively obtained by hydrolysis of *exo*-**14** with aqueous $NaHCO_3$ in 95% yield. Suitable crystals for X-ray analysis were grown of this compound proving its *exo*-configuration (Fig. 2).

Table 1. Product distribution in the reaction of fluoromaleic anhydride (**13**) with furan



13 *exo*-**14** *exo*-**15** *endo*-**15**

Condition	Yield (%)		
	14	*exo*-**15**	*endo*-**15**
55°C	70	11	1
Microwaves	37	29	4

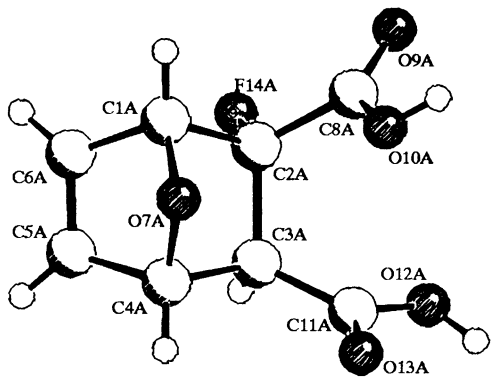
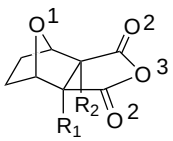
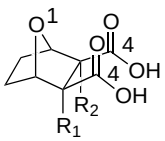


Figure 2. Crystal structure analysis of *exo*,*exo*-2-fluoro-7-oxa-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid (*exo*-**15**).[22]

Table 2. ^{17}O NMR shifts of fluorinated cantharidin analogues **5**–**7** and their non-fluorinated parent compounds **1**–**3**



1: R_1, R_2= CH_3
2: R_1, R_2= H
5: R_1= F, R_2= H



3: R_1, R_2= H
6: R_1, R_2= F
7: R_1= F, R_2= H

Compound	^{17}O NMR shift[33] of functional group in position			
	1	2	3	4
1[34]	86.4	362.2	299.9	–
2	81.6	371.8	304.1	–
5	79.2	377.0	302.2	–
3	83.4	–	–	260.0
6	72.1	–	–	259.9
7	87.7	–	–	257.3

The final step to 2-fluoronorcantharidin (**5**)[27] consists of conventional hydrogenation (H_2 over Pd/C) of **14** in 92% yield (Scheme 2). As a byproduct, the hydrogenated diacid 2-fluoroendothall (**7**) was obtained (7%). This route represents the first access to the hitherto unknown **5** in four steps and an overall yield of 29% from **11**. Compound **5** was converted to 2-fluoroendothall (**7**) in 93% yield by mild basic hydrolysis with aqueous $NaHCO_3$ or in a slow fashion by simply exposing **5** to air.

^{17}O NMR measurements. In order to evaluate the influence of the fluorine substituent(s) in the new compounds **5**–**7**, we undertook ^{17}O NMR measurements. Concerning ^{13}C NMR spectroscopy, halogens (among others) are known to exert a dihedral angle dependent highfield effect in γ-position.[28] The γ-effect is also known in ^{17}O NMR spectroscopy.[29] As the oxygens are likely to bind to the active site of enzymes (vide supra), altered interaction due to changed electronic properties can be expected with fluorinated analogues of cantharidin (**1**) and norcantharidin (**2**), respectively. In order to be able to evaluate the ^{17}O NMR measurements, the non-fluorinated parent compounds of **5**–**7**, norcantharidin (**2**) and endothall (**3**) were synthesized for means of comparison according to literature procedures.[30] The results are shown in Table 2. Although chemical shifts of compounds **5** and **7** do not differ significantly from their parent compounds **2** and **3** within the uncertainty of this NMR method, **6** shows a highfield shift of ca. −11 ppm compared to **3** for the bridging oxygen (position 1) hinting at an electron donating effect of the fluorine substituents of **6**. Regarding cantharidin (**1**), **5** shows a downfield shift of ca. +15 ppm for the carbonyl oxygens (position 2). All other effects are smaller (Table 2). Known γ-effects in ^{17}O NMR spectroscopy range between −2 and −11 ppm.[29] Concerning fluorinated compounds, just a few cases are studied where fluorine substituents exert a γ-effect upon oxygen atoms: all studies dealt either with trifluoromethyl compounds (highfield shift −8 to −29 ppm compared to the non-fluorinated parent compounds)[29,31] or with monofluorinated aromatic compounds (−19 to −25 ppm).[32]

Conclusion. The first fluorinated cantharidin derivatives **4**–**7** were prepared via short synthetic pathways with Diels–Alder reactions as the key step. ^{17}O NMR measurements suggest that **6** possesses increased electron density compared to **3** at the bridging oxygen at least. On the other hand, the carbonyl oxygens of compound **5** exhibit decreased electron density with respect to **1**.

Acknowledgements

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21. Compound **6**: Sublimation: 170–172°C. **^{1}H NMR** (300 MHz, CD_3OCD_3): δ 1.67–1.80 (m, 2H), 2.00–2.13 (m, 2H), 4.92 (dd, $J=3.6$, 2.4 Hz, 2H), 10.38 (br s, 2H). **^{13}C NMR** (75 MHz, CD_3OCD_3): δ 22.9 (tt, $J=3.2$ Hz), 80.3 (dt, $J=10.8$ Hz), 93.5 (dd, $J=222.0$ Hz, 18.5 Hz), 167.5 (dd, $J=16.5$ Hz, 12.7 Hz). **^{19}F NMR** (282 MHz, CD_3OCD_3): δ −167.0 (s). **^{17}O NMR** (49 MHz, CH_3CN, 333K): δ 72.1 (1O), 259.9 (4O). **GC/MS** (of the TMS-ester): m/e (%): 351 (10) [M^+-CH_3], 297 (14), 256 (8), 249 (20), 221 (26), 187 (10), 185 147 (26), 77 (42), 73 (100), 45 (9). Anal. calcd for $C_8H_8F_2O_5$: C, 43.25, H, 3.63; found: C, 43.36; H, 3.86.
22. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 164387 and 164388. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 1223-336033 or e-mail: deposit@ccdc.cam.ac-uk].
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27. Compound **5**: **^{1}H NMR** (300 MHz, CD_3OCD_3): δ 1.75–2.01 (m, 3H), 2.07–2.21 (m, 1H), 3.58 (d, $J=13.1$ Hz, 1H), 4.92–4.96 (m, 1H), 4.97–5.01 (m, 1H). **^{13}C NMR** (75 MHz, CD_3OCD_3): δ 22.7 (dt, $J=5.1$ Hz), 27.3 (t), 54.0 (d, $J=21.6$ Hz), 82.6 (dd, $J=3.8$ Hz), 80.4 (dd, $J=24.2$ Hz), 98.5 (d, $J=216.2$ Hz), 168.9 (d, $J=26.7$ Hz), 168.6 (d, $J=7.6$ Hz). **^{19}F NMR** (282 MHz, CD_3OCD_3): δ −183.0 (d, $J=13.1$ Hz). **^{17}O NMR** (49 MHz, CH_3CN, 333K): δ 79.2 (1O), 302.2 (1O), 377.0 (2O). **GC/MS** m/e

(%): 186 (3) [M^+], 158 (18), 140 (8), 118 (35), 114 (9), 99 (12), 86 (100), 70 (20), 69 (59), 57 (12), 51 (6), 39 (8). Exact mass: $C_8H_7O_4F$ requires 186.0328, found: 186.0307.

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33. Spectra were recorded on a Bruker AM 360 in acetonitrile at 333 K; external standard: dioxane (0.0 ppm). All spectra were processed by NMR software in exactly the same manner.
34. Compound **1** is commercially available.