Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1267–1271

Probing structure/affinity relationships for the *Plasmodium falciparum* hexose transporter with glucose derivatives

Martine Fayolle,[a] Marina Ionita,[a] Sanjeev Krishna,[b] Christophe Morin[a,*] and Asha Parbhu Patel[b]

[a]*Laboratoire d'Etudes Dynamiques et Structurales de la Sélectivité (LEDSS), UMR 5616 (IFR 2607), Université Joseph Fourier Grenoble 1, 38402 St Martin d'Hères, France*
[b]*St. George's University of London, Centre for Infection, Division of Cellular and Molecular Medicine, Cranmer Terrace, London SW17 ORE, UK*

Received 28 October 2005; revised 17 November 2005; accepted 18 November 2005
Available online 19 December 2005

Communicated by Stephen Neidle

Abstract—A series of 3-O-substituted glucose derivatives was prepared with alkyl, alkenyl, aromatic and ferrocenic substituents; to vary lipophilicity and hydrogen bonding ethylenedioxy and perfluorinated fragments were also introduced. Apparent affinities for the *Plasmodium falciparum* hexose transporter (PfHT) were determined after heterologous expression in *Xenopus* oocytes, with highest affinities for compounds with C8–C13 lipophilic chains. As no derivatives show significant affinity for the mammalian glucose transporter (GLUT1), these structure/affinity assays contribute to design of potent PfHT inhibitors and eventual development of antimalarials.

In view of the increasing resistance to conventional antimalarials, new drugs and new targets are urgently being sought for malaria.[1,2] *Plasmodium falciparum* depends on a continuous supply of glucose from its mammalian host for its energy requirements during asexual replication. During this stage of its life-cycle, *P. falciparum* grows and multiplies within red cells. In comparison with uninfected red cells, parasites increase utilisation of glucose by up to 100 times. Delivery of this glucose to the intraerythrocytic parasite is mediated by PfHT, a parasite-encoded facilitative transporter that is localised to the region of the parasite plasma membrane.[3,4] Heterologous expression of PfHT in *Xenopus laevis* oocytes has allowed studies on its substrate specificity as well as comparisons with the major mammalian hexose transporter, GLUT 1, which is found on the surface of erythrocytes. Mammalian GLUTs are usually specialised to transport either glucose or fructose, whereas PfHT can mediate uptake of both substrates. These studies have also established that some 3-*O* derivatives of glucose can selectively inhibit PfHT and rapidly disrupt intraparasitic homeostasis.[5] Exposure of parasites in culture to one derivative (CM3361, see structure **1**) kills parasites in concentrations that do not disrupt host red cells, validating PfHT as a new drug target and opening up a new approach to identify antimalarial agents.[2] The aim of this work was to extend our insights into structure/affinity relationships of substrates that may interact with PfHT, so that results can be used to inform improvements in design of antimalarials aimed at PfHT.

Evidence that **1**, a glucose derivative in which an undecenyl side chain is attached to *O*-3, inhibits PfHT[5] was the starting point from which to explore several aspects:

- Is the methylene chain of **1** of an optimal length?
- Would reduced compounds (i.e., without double bonds) retain comparable affinities?
- What would be the effects of changes in the lipophilicity/hydrophilicity?

To address these questions, we have prepared glucose derivatives of different types (**3**–**17**, **21**–**29**, **32**–**34**, **37**

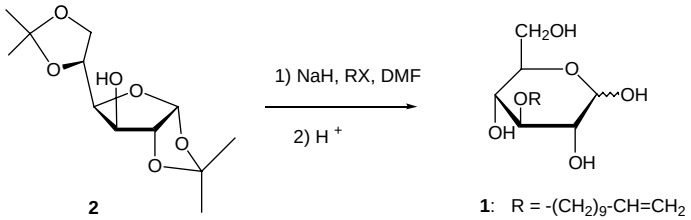
Keywords: Glucose; Carbohydrate transporters; PfHT; Inhibitors; Malaria.
* Corresponding author. E-mail: christophe.morin@ujf-grenoble.fr

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.11.068

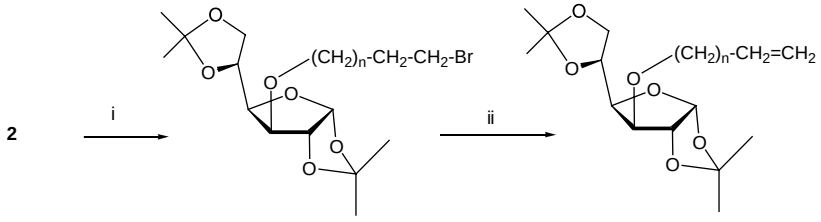
and **40**) and determined their apparent affinities for PfHT.

3-*O*-Glucose derivatives can be obtained, as depicted in general Scheme 1, by alkylation of the sodium salt of diacetone-D-glucose (**2**), followed by deprotection of the acetals. The synthesis of alkyl derivatives **3**–**11**[6] is straightforward since the required bromides are commercially available. To obtain the lower (**12**) and higher (**14** and **15**) homologues of **1**, it was found advantageous to mono-alkylate first **2** with α,ω-dibromoalkanes and then to generate the double bond by base-promoted elimination of the remaining bromide (Scheme 2). For the synthesis of **13**, the alkylating agent was derived[7] from commercially available dec-9-en-1-ol. Derivatives **16** and **17** in which the unsaturation lies within the chain were also considered; to prepare **16**, the required bromide was obtained by alkylation[8] of dec-1-yne with 1-6-dibromohexane; for the preparation of **17**, *threo*-aleuritic acid (**18**) was converted into alkene **19**,[9] which was then transformed into ω-iodo-ester **20**[10] (Scheme 3) with which **2** was alkylated (Scheme 1). Three analogues bearing a phenyl group were also prepared: in **21** and **22** at the terminal position, and in **23** on the chain, using relevant bromides.[11,12] Ferrocenyl groups[13] were also introduced (**24** and **25**) after alkylation of **2** with the appropriate ferrocenyl bromides,[14] which was followed by cleavage of the acetals (Scheme 1).

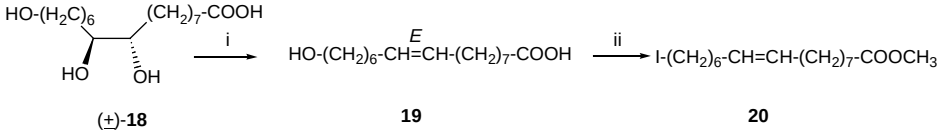
To increase the hydrophilicity of the methylene chain, analogues of **1** (i.e., **26**–**29**) in which one or two oxygen atoms are in the chain were prepared. 1,7-Heptanediol was mono-O-allylated and the remaining hydroxyl group converted into an iodide[15] (Scheme 4); its reaction with **2** was followed by deprotection of the acetals, which afforded **26**. For the synthesis of **27**, **2** was first converted to the hydroxyethyl derivative **30**,[16] which was alkylated with ω-bromo-hept-1-ene and deprotected. For the preparation of **28** and **29**, since there is an oxygen atom β to the leaving group,[17] triflate **31** was selected as the alkylating agent and was prepared from *O*-allyl-ethylene glycol[18] (Scheme 4). Compound **31** was used to alkylate **2** and **30**, which afforded **28** and **29**, respectively, after acidic cleavage of the acetals.



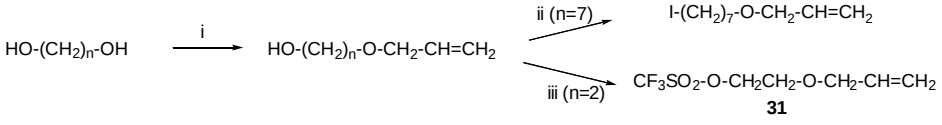
Scheme 1. Preparation of 3-*O*-substituted-D-glucose.



Scheme 2. Reagents and conditions: (i) 1—NaH, DMF; 2—Br-$(CH_2)_n$-CH_2CH_2-Br (1.2 equiv); (ii) K-*tert*-butylate, THF, 50 °C, 4 h.



Scheme 3. Reagents and conditions: (i) triethylorthoformate see Ref. 9; (ii) 1—CH_3COCl–CH_3OH—67%; 2—MsCl–Et_3N—98%; 3—NaI, acetone, overnight reflux 94%.



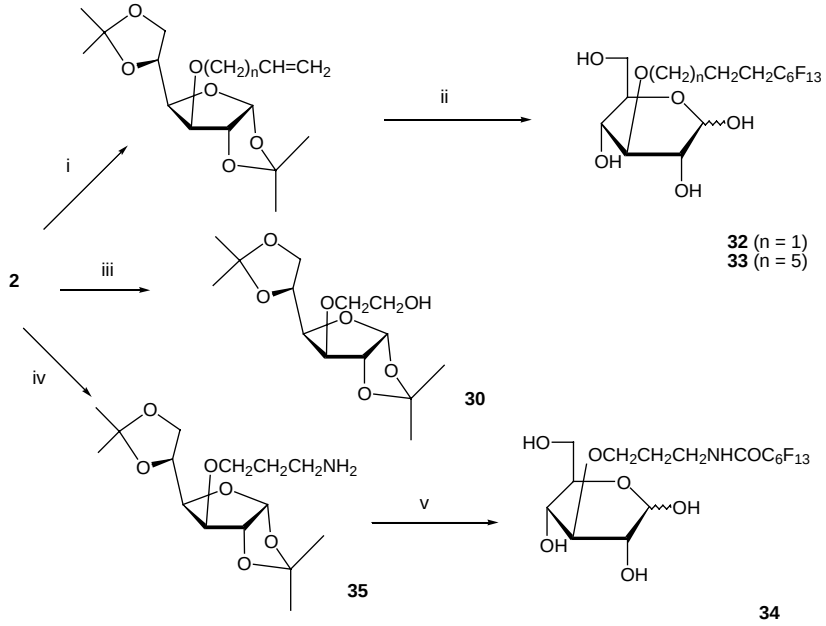
Scheme 4. Reagents and conditions: (i) NaH, allyl bromide, see Ref. 15; (ii) 1—TsCl (85%); 2—NaI (90%); (iii) Tf_2O (1 equiv), diisopropylethyl amine (0.95 equiv), CH_2Cl_2, −20 °C, 57%.

In view of the profound changes in lipophilicity and hydrogen bonding brought about by introduction of fluorinated fragments,[19] fluorinated derivatives were prepared; **32** and **33** were obtained after the addition[20] of perfluorohexyl iodide to alkenyl derivatives (Scheme 5) followed by reduction, whereas **34** was obtained by acylation of amine **35**[21,22] with perfluoroheptanoyl chloride.[23] These reactions were followed by cleavage of the acetals.

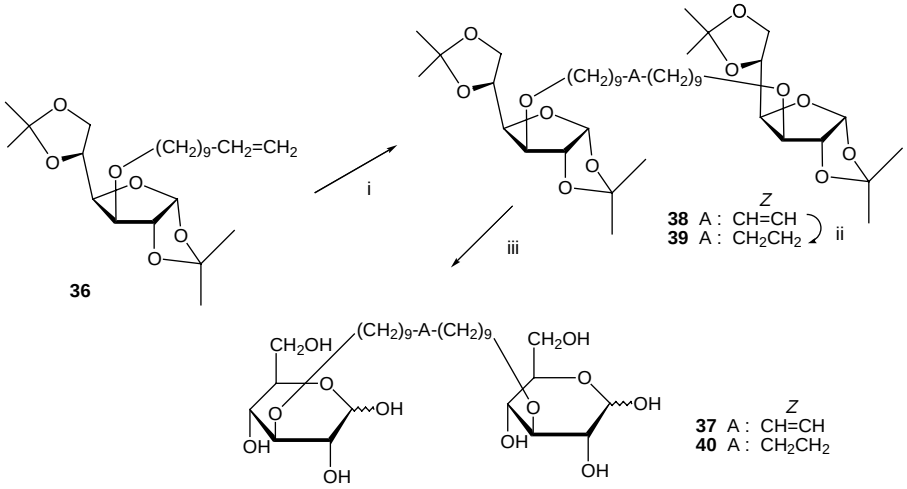
Finally, the preparation of a 'dimer' of **1** (see structure **37** in which two glucose units are linked by a 20-carbon chain in a symmetrical way) was considered. To obtain the same, **36** was subjected to cross-metathesis, using type-II Grubbs catalyst[24] which led to **38** (Scheme 6); the configuration of the double bond was determined as *Z* after the observation of a large coupling constant ($^3J_{-CH=CH-}$ = 15.8 Hz) in the ^{13}C–^1H satellite peaks.[25] The saturated analogue **39** was obtained by hydrogenation, and both compounds were submitted to acid treatment, which yielded **37** and **40**, respectively.

All the prepared analogues (except for **11**, which was not soluble enough) were tested for their ability to inhibit uptake of D-glucose mediated by PfHT expressed in *Xenopus* oocytes. Apparent inhibitory constants were derived after curve fitting (Prism, Graphpad v4.0) using parameters for one-site competition and the table displays affinities for PfHT.

At the onset of this work, it was known that affinities for PfHT of 'short chain' analogues of **1** (i.e., O-3 methyl, ethyl, benzyl and hydroxyethyl derivatives)[5] were low (K_i in the 1–15 mmol range) and even lower compared



Scheme 5. Reagents and conditions: (i) NaH, $Br(CH_2)_nCH{=}CH_2$; (ii) 1—I-C_6F_{13}, triphenylphosphine; 2—LAH (n = 1: see Ref. 20); 3—H^+; (iii) 1—$BrCH_2COOC_2H_5$; 2—LAH see Ref. 16; (iv) 1—NaH, DMF, Br-$(CH_2)_3$-NH-Cbz—58%; 2—H_2, Pd/C; (v) 1—$C_6F_{13}COCl$, Huning's base, 4 days—43%; 2—H^+.



Scheme 6. Reagents and conditions: (i) Grubbs type-II catalyst, CH_2Cl_2, 40 °C, 48 h—59%; (ii) H_2–Pd/C; (iii) H^+.

Table 1. K_i/inhibition test for D-glucose uptake of various 3-*O*-D-glucose derivatives against PfHT

Compound	R (O-3-substituent)	K_i[a] (mM)
1	$-(CH_2)_9-CH=CH_2$	0.053 ± 0.019
3	$-(CH_2)_6-CH_3$	<0.5
4	$-(CH_2)_7CH_3$	0.030; 0.049[b]
5	$-(CH_2)_8-CH_3$	0.035; 0.032[b]
6	$-(CH_2)_9-CH_3$	0.029; 0.019[b]
7	$-(CH_2)_{10}-CH_3$	0.023 ± 0.002
8	$-(CH_2)_{11}-CH_3$	0.030 ± 0.008
9	$-(CH_2)_{13}-CH_3$	>0.5
10	$-(CH_2)_{17}-CH_3$	>0.5
11	$-(CH_2)_{19}-CH_3$	[c]
12	$-(CH_2)_7-CH=CH_2$	0.041 ± 0.002
13	$-(CH_2)_8-CH=CH_2$	0.049 ± 0.004
14	$-(CH_2)_{10}-CH=CH_2$	0.036 ± 0.004
15	$-(CH_2)_{13}-CH=CH_2$	0.037 ± 0.009
16	$-(CH_2)_8-X-(CH_2)_7-COOCH_3$[d]	>0.5
17	$-(CH_2)_6-CH=CH-(CH_2)_7-CH_3$[e]	>0.5
21	$-(CH_2)_5-C_6H_5$	0.072; 0.064[b]
22	$-(CH_2)_8-C_6H_5$	0.148 ± 0.026
23	$-(CH_2)_5-C_6H_4-(CH_2)_3-CH_3$[f]	0.081
24	$-(CH_2)_6-Fc$[g]	>0.5
25	$-(CH_2)_{11}-Fc$[g]	NI
26	$-(CH_2)_7-O-CH_2-CH=CH_2$	>0.5
27	$-(CH_2)_2-O-(CH_2)_7-CH=CH_2$	0.590 ± 0.072
28	$-(CH_2)_2-O-CH_2-CH=CH_2$	>0.5
29	$-(CH_2)_2-O(-CH_2)_2-O-CH_2-CH=CH_2$	NI
32	$-(CH_2)_3-(CF_2)_5-CF_3$	>0.5
33	$-(CH_2)_7-(CF_2)_5-CF_3$	0.13[h]
34	$-(CH_2)_3-NHCO-(CF_2)_5-CF_3$	NI
37	Unsaturated 'dimer'[i]	0.25; 0.14[b]
40	Saturated 'dimer'[i]	0.56; 0.54[b]

[a] Values are means of three experiments (standard error of the mean given) unless otherwise noted.
[b] Two experiments (i.e., two individual values).
[c] Too insoluble for assay. NI no inhibition.
[d] X denotes triple bond.
[e] Configuration of double bond is *E*.
[f] *para*-Isomer.
[g] Fc stands for ferrocene.
[h] Insufficient material to carry out repeats.
[i] For structures, see Scheme 6.

with D-glucose; this led us to consider higher homologues and hence the derivatives presented in the Table 1. As affinities in the 20–50 μM range are recorded for **4**–**7** (alkyl) and **12**–**15** (alkenyl) C8–C13 derivatives but not for longer-chain derivatives (i.e., **9**–**11**, **16** and **17**), this clearly shows that the chain length of the substituent is important. A comparison of compounds with the same substituent length but differing in the presence of a terminal double bond (**7** vs **1**, **5** vs **12**, **6** vs **13** and **8** vs **14**) shows minimal consequences; however, in the case of a longer chain (**9** vs **15**), the affinity is restored when a terminal double bond is introduced. With regard to 'dimers' **37** and **40** (which can be viewed as two hydrophilic groups linked by a lipophilic chain) there is a ca. 10-fold decrease in their affinities when compared to that of **1** (the parent compound). The introduction of an aromatic ring, whether in the end position (**21** and **22**) or not (**23**), decreases the affinity as well, whereas the presence of ferrocenyl groups (**24** and **25**) results in loss of inhibition. Perfluorinated derivatives (**32** and **34**) do not exhibit interaction with PfHT, or interact poorly (**33**), presumably through the mechanism of increased hydrophobicity brought about by fluorinated fragments—fluorine being a polar hydrophobic element.[19] Conversely, it is noteworthy that when hydrophilicity is increased, as in compounds **26**–**29** in which an oxygen replaces carbon atoms in the chain,[26] affinity is also decreased. All these data show that a C8–C13 lipophilic chain should be present in the substituent for it to inhibit PfHT-mediated glucose uptake in oocytes, which is the main conclusion which can be drawn from the affinity measurements.

Importantly, none of the derivatives displayed in the table inhibited GLUT1 (the ubiquitous human glucose transporter) at concentrations effective against PfHT and the selectivity of **1** and congeners for PfHT thus appears to be a salient feature of 3-O-substituted glucose derivatives. Since the presence of a C8–C13 lipophilic chain[27] correlates with inhibition, our structure–function analyses of the molecular requirements for inhibition of the critical hexose transporter of *P. falciparum* reinforce the 'lollypop' model previously put forward.[5] This will hopefully assist in the design of more potent inhibitors that can be used as templates for drug design of carbohydrate-based antimalarials.

References and notes

1. For reviews, see inter alia: (a) Ridley, R. G. *Nature* **2002**, *415*, 686; (b) Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 5274; (c) Go, M.-L. *Med. Res. Rev.* **2003**, *23*, 456; (d) Delhaes, L.; Benoit-Vical, F.; Camus, D.; Capron, M.; Meunier, B. *Drugs* **2003**, *6*, 674; (e) Waters, N. C.; Dow, S. G.; Kozar, M. P. *Expert Opin. Ther. Pat.* **2004**, *14*, 1125; (f) Staines, H.; Ellory, J. C.; Chibale, K. *Comb. Chem. High Throughput Screening* **2005**, *8*, 81.
2. Joët, T.; Morin, C.; Fischbarg, J.; Louw, A. I.; Eckstein-Ludwig, U.; Woodrow, C.; Krishna, S. *Expert Opin. Ther. Targets* **2003**, *7*, 593.
3. Woodrow, C. J.; Penny, J. I.; Krishna, S. *J. Biol. Chem.* **1999**, *274*, 7272.
4. Woodrow, C. J.; Burchmore, R. J. S.; Krishna, S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9931.
5. Joët, T.; Eckstein-Ludwig, U.; Morin, C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7476.
6. For the preparation of *O*-3 alkyl glucose, see, for example: Ikegawa, T.; Irinoda, K.; Saze, K.; Katori, T.; Matsuda, H.; Ohkawa, M.; Kosik, M. *Chem. Pharm. Bull.* **1987**, *35*, 2894.
7. Fryxell, G. E.; Rieke, P. C.; Wood, L. L.; Engelhard, M. H.; Williford, R. E.; Graff, G. L.; Campbel, A. A.; Wiacek, R. J.; Lee, L.; Halverson, A. *Langmuir* **1996**, *12*, 5064.
8. Kates, M. J.; Schaube, J. H. *J. Org. Chem.* **1996**, *61*, 4164.
9. Singh, A. N.; Mhaskar, V. V.; Dev, S. *Tetrahedron* **1978**, *34*, 595.
10. For another synthesis, see: Subramanian, G. B. V.; Ahua, S. *Indian J. Chem.* **1996**, *35B*, 724.
11. α,ω-Dibromoalkanes with phenyllithium: Spencer, T. A.; Onafrey, T. J.; Cann, R. O.; Russel, J. S.; Lee, L. E.; Blanchard, D. E.; Castro, A.; Gu, P.; Jiang, G.; Shechter, I. *J. Org. Chem.* **1999**, *64*, 807.
12. α,ω-Dibromoalkanes with *p*-butylphenylmagnesium bromide: Nishimura, J.; Yamada, N.; Horiuchi, Y.; Ueda, E.;

Onbayashi, A.; Oku, A. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2035.
13. For a review about antimalarials based on ferrocene, see: Biot, C. *Curr. Med. Chem.* **2004**, *3*, 1.
14. Yu, C. J.; Wang, H.; Wang, Y.; Yowanto, H.; Kim, J. C.; Donilon, L. H.; Tao, C.; Strong, M.; Chong, Y. *J. Org. Chem.* **2001**, *66*, 2937.
15. The intermediate tosylate is a known compound: Toepfer, A.; Ktretzchmar, G.; Schuth, S.; Sonnetag, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1317.
16. Bignan, G.; Morin, C.; Vidal, M. *Carbohydr. Res.* **1993**, *248*, 371.
17. β-Iodo-ethers are reluctant to displacement reactions (see Ref. 16 and refs. therein) and this peculiar stability has resulted in their use for radio-labelling; see: (a) Hamant, S.; Mathieu, J.-P.; Morin, C.; Trimcev, I.; Vidal, M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1687; (b) Rossouw, D. D.; Coenen, H. H. *Nucl. Med. Biol.* **2003**, *30*, 373.
18. Lee, G. H.; Choi, E. B.; Lee, E.; Pak, C. S. *J. Org. Chem.* **1996**, *59*, 1428.
19. For recent reviews: (a) Biffinger, J. C.; Kim, H. W.; DiMagno, S. *ChemBioChem* **2004**, *5*, 622; (b) Böhm, H.-J.; Banner, D.; Kansy, M.; Kuhn, B.; Müller, K.; Stahl, U.; Obst-sander, M. *ChemBioChem* **2004**, *5*, 637.
20. (a) Huang, W.-Y.; Zhang, H.-Z. *J. Fluorine Chem.* **1990**, *50*, 133; (b) Lumbierres, M.; Moreno-Manas, M.; Valltibera, A. *Tetrahedron* **2002**, *58*, 4061; (c) Yu, B.; Li, B.; Zhang, J.; Hui, Y. *Tetrahedron Lett.* **1998**, *39*, 4871.
21. Compound **34** was obtained by alkylation of **2** with *N*-benzyloxy-carbonyl-3-bromopropylamine (prepared according to Robarge, M. J.; Husbands, S. M.; Kyieltyka, A.; Brodbeck, R.; Thurkauf, A.; Newman, A. H. *J. Med. Chem.* **2001**, *44*, 3175,) and reduction.
22. For another preparation, see: Corbett, W. M. *J. Chem. Soc.* **1961**, 2926.
23. Afzal, J.; Fung, B.-M.; O'Real, E. A. *J. Fluorine Chem.* **1987**, *34*, 385.
24. Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360.
25. In R–^{13}CH=^{12}CH–R, due to the presence of differing carbon isotopes, the symmetry is disrupted, which allows the observation of coupling constants. We thank J. Garcia for theoretical simulation of the spectra of *E* and *Z* isomers.
26. The presence of ethylenedioxy fragments is known to increase aqueous solubility.
27. Of interest are the recent results (Liu, Y.; Wong, V. K.- W.; Ko, B. C.-B.; Wong, M.-K.; Che, C.-M. *Org. Lett.* **2005**, *7*, 1561), obtained with artemisinin derivatives in which introduction of lipophilic chains resulted in an up to 200-fold increase in activities.