Terpenoids

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Improving Physical Properties via C–H Oxidation: Chemical and Enzymatic Approaches**

*Quentin Michaudel, Guillaume Journot, Alicia Regueiro-Ren, Animesh Goswami, Zhiwei Guo, Thomas P. Tully, Lufeng Zou, Raghunath O. Ramabhadran, Kendall N. Houk, and Phil S. Baran**

Abstract: *Physicochemical properties constitute a key factor for the success of a drug candidate. Whereas many strategies to improve the physicochemical properties of small heterocycle-type leads exist, complex hydrocarbon skeletons are more challenging to derivatize because of the absence of functional groups. A variety of C–H oxidation methods have been explored on the betulin skeleton to improve the solubility of this very bioactive, yet poorly water-soluble, natural product. Capitalizing on the innate reactivity of the molecule, as well as the few molecular handles present on the core, allowed oxidations at different positions across the pentacyclic structure. Enzymatic oxidations afforded several orthogonal oxidations to chemical methods. Solubility measurements showed an enhancement for many of the synthesized compounds.*

The rates of attrition for small-molecule drug candidates remain excessively high at any stage of drug development despite a continuously improving understanding of drug–target interactions, as well as drug distribution and metabolism.[1] Consequently, the cost of discovering and developing a drug in 2010 was estimated to be approximately $1.8 billion.[2] Historically, some of the main reasons behind drug failure are the lack of efficacy, toxicity, and poor pharmacokinetic properties or bioavailability. Unsatisfactory physicochemical properties have specifically emerged as a common source of drug failure.[3] A subtle balance between lipophilicity and polarity is therefore required to ensure a viable future for a drug candidate. In the case of heterocyclic leads, this balance can be achieved by substituting some positions of the heteroarenes with hydrogen-bond donors and/or acceptors, as well as various fluorinated motifs. When the drug candidate is a natural product derivative, however, the quest for an ideal solubility and cell permeability is often hampered by the few positions which can be accessed. This is especially true in the case of lowly oxidized terpenes, where only a few carbon atoms of the skeleton can be modified using conventional chemical means. Our laboratory has been developing various aliphatic C–H functionalization methods and strategies for a biomimetic 'oxidase phase' in natural-product synthesis.[4] It was in this context that a collaboration was forged with Bristol-Myers Squibb to probe the applicability of these studies in drug discovery and more precisely the improvement of drug physicochemical properties. The lupane natural products betulin (**1**) and betulinic acid (**2**) are ideal substrates to explore this concept because of their promising in vitro bioactivity coupled with their extreme insolubility, thus limiting their medicinal potential (Figure 1). Outlined herein is a strategy for improving the solubility of this promising natural-product class using both chemical and enzymatic means.[5] This combination of orthogonal techniques has led to a dramatic improvement in solubility of the lupane core (Figure 1), thus setting the stage to realize their full medicinal potential.

Betulin (**1**) and betulinic acid (**2**) are two natural pentacyclic triterpenes isolated from the bark of the birch tree and only differ by the oxidation at C28 (Figure 1).[6] Betulin is more abundant than its carboxylic acid counterpart but is generally less bioactive.[7] Several methods have been developed to convert **1** into **2**.[8] Betulinic acid displays many intriguing pharmacological properties such as anti-inflammatory, anticancer, and anti-HIV, the latter two being the most promising for pharmaceutical applications.[7,9] However, the low solubility of **2** in H_2O constitutes a serious limitation for its use as a therapeutic, since it would make formulation for oral delivery difficult.[7] To render **1** and **2** more soluble in aqueous media, the lipophilic carbon skeleton must be decorated with heteroatoms. However, oxidation of the lupane core presents many challenges because of the very few functional groups present on the skeleton. Prior art has mainly focused on oxidation of the A ring that already possesses an alcohol group at C3 as a handle,[10] and modification of the isopropenyl group by allylic C–H oxidation or direct transformation of the alkene.[10c,11] In

[*] Q. Michaudel, Dr. G. Journot, Prof. Dr. P. S. Baran
Department of Chemistry, The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
E-mail: pbaran@scripps.edu
Dr. A. Regueiro-Ren
Discovery Chemistry, Bristol-Myers Squibb
5 Research Parkway, Wallingford, CT 06492 (USA)
Dr. A. Goswami, Dr. Z. Guo, Dr. T. P. Tully
Chemical Development, Bristol-Myers Squibb
One Squibb Drive, New Brunswick, NJ 08903 (USA)
Dr. L. Zou, Dr. R. O. Ramabhadran, Prof. Dr. K. N. Houk
Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90095-1569 (USA)

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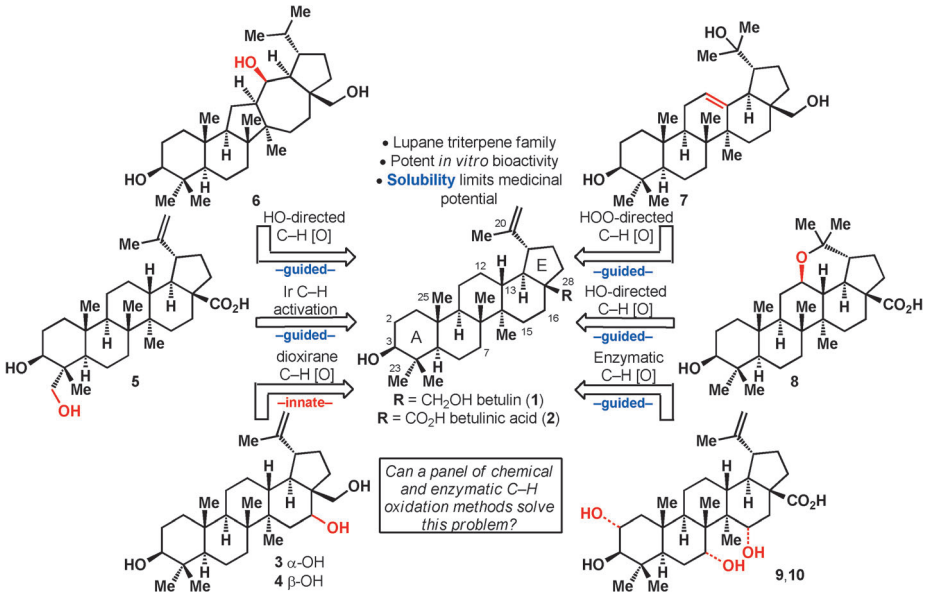


Figure 1. Diversification of the lupane core by C–H oxidation.

contrast, known oxidations of the B, C, and D rings are scarce and have mostly utilized the C28 alcohol as a directing group.[12] To the best of our knowledge, only one group has reported a nondirected oxidation of lupanes, leading to functionalization of the C ring.[13] However, the reaction conditions (CrO_3 in boiling acetic acid) afforded many compounds in yields all below 3%.[13a,b] Finally, it is of note that several C–H bonds can be biochemically oxidized by feeding **2** to microorganisms.[7a]

With that precedent in mind, and by analogy to studies conducted on eudesmane terpenes,[4a] two different strategies were applied to oxidize various unactivated aliphatic C–H bonds of **1** or **2**. Thus, nondirected (innate) C–H oxidation would capitalize on the default reactivity of the 48 C–H bonds of **1** (46 for **2**), whereas directed (guided) C–H oxidation would employ the hydroxy groups at C3, C28, or C20 (arising from hydration of the alkene).[14] To achieve innate C–H oxidation, the two hydroxy groups and the alkene required protection, since they are the most prone to oxidation. Carreira's hydration conditions[15] followed by diacetylation afforded substrate **12** in good yield (Figure 2B). X-ray crystallographic analysis of **12** suggested that it would be oxidized at a methylene position since all the methine positions are fairly hindered. The electronic character of all the C–H bonds was evaluated through ^{13}C NMR studies.[4a,16] Every carbon peak was unambiguously assigned using ^{13}C–^{13}C INADEQUATE NMR spectroscopy and the chemical-shift trend was determined as follows (see the Supporting Information for actual values):

$$\delta_{C6} < \delta_{C11} < \delta_{C2} < \delta_{C15} \approx \delta_{C21} \approx \delta_{C12} \approx \delta_{C16} < \delta_{C22} \approx \delta_{C7} < \delta_{C1} \ll \delta_{C28}$$

Based on this analysis, the C6 to C16 positions are the most electron-rich methylenes, and thus most likely to be oxidized. Dozens of oxidants were screened, but most of them resulted in no reaction or an inextricable mixture of products, including the reported CrO_3 conditions.[13a,b] Fortunately, it was found that methyl(trifluoromethyl)dioxirane (TFDO) afforded the C16 ketone product **14** in 30% yield as a major product, as confirmed by X-ray crystallography. No minor products were isolated in quantities sufficient for characterization, but it is of note that none of these products contained a ketone motif. While TFDO has been previously used to selectively oxidize a sesquiterpene skeleton in the total synthesis of eudesmantetraol,[4a] this reaction constitutes the first example of selective methylene oxidation in the context of a complex triterpene substrate. The striking selectivity for C16 cannot be explained solely by electronic factors since six carbon atoms are more electron-rich based on ^{13}C NMR spectroscopy.

Calculations using the UB3LYP functional were performed to determine the origins of this surprising selectivity. Activation energies for attack at the ring carbon hydrogen bonds by dimethyldioxirane (DMDO) were computed (Figure 3A). Based on our previous computational studies, C–H oxidation using DMDO or TFDO involves a concerted reaction, initiated by C–H abstraction followed by a barrierless oxygen rebound from a radical pair.[17] DMDO is less reactive than TFDO and more selective.[17] The calculated $\Delta H^{\ddagger}$ value for the oxidation of the equatorial C16–H is found to be 3.5 kcal mol^{-1} lower than that of C6–H and is considerably lower than activation energies computed for all other positions (see Figure 3A). This difference drops to 1.6 kcal mol^{-1} with TFDO (see the Supporting Information). The abstraction of the C16 equatorial hydrogen atom is consistent with our previous finding that equatorial C–H bonds are more reactive because of both the strain-release effect[17,18] and higher steric accessibilities. Thus, Figure 3B shows an oxygen atom of TFDO just touching the surface of the hydrogen at C16 and C6. The former occurs with no clashing of van der Waals surfaces, while approach at C6–H involves significant steric repulsions of the dimethyl group at C4 of betulin and the CF_3 groups of TFDO. These interactions are confirmed in the transition state geometries given in Figure S13 in the Supporting Information. The stability of radicals formed by hydrogen abstraction at all positions were also calculated (Figure 3A). Remarkably, the C–H oxidation process is extremely selective, and hydrogen abstraction occurs only at C16. This selectivity is present despite the fact that the diradical transition state has appreciable diradical character at C16 in **12**, where a radical is intrinsically of low stability. Finally, calculations on an analogue of **12** where the C17 CH_2OAc group is replaced by

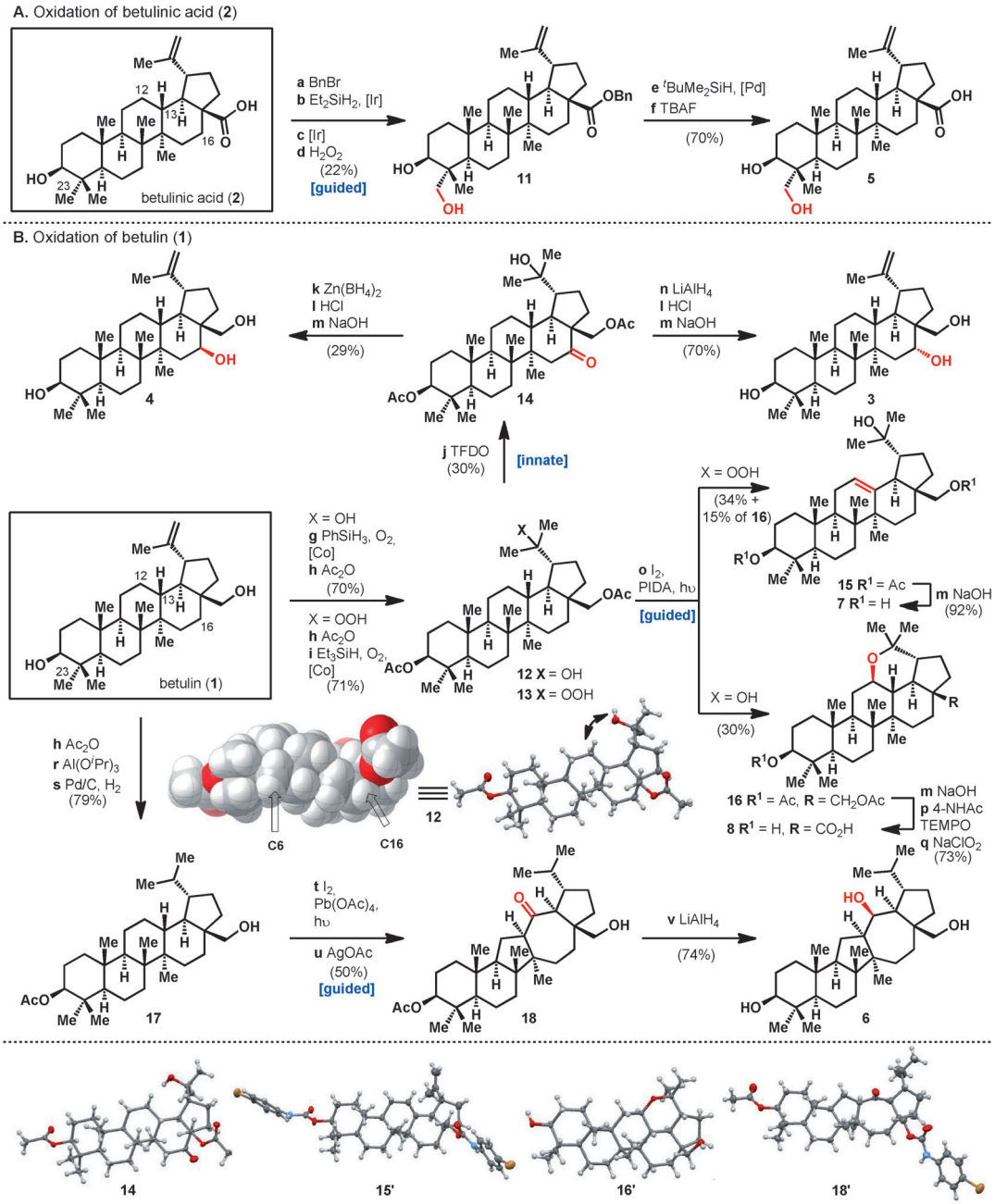


Figure 2. Divergent synthesis of oxidized compounds from a) betulinic acid and b) betulin. Reagents and conditions: a) BnBr, K_2CO_3, 60 °C, 3.5 h, 92%; b) 1 mol% [{Ir(cod)OMe}$_2$], Et_2SiH_2, RT, 12 h; c) 2 mol% [{Ir(cod)OMe}$_2$], 5 mol% Me_4Phen, norbornene, 120 °C, 36 h; d) H_2O_2, $KHCO_3$, 50 °C, 12 h, 24% (over 3 steps); e) 50 mol% $Pd(OAc)_2$, Et_3N, *t*$BuMe_2SiH$, 60 °C, 2.5 h; f) TBAF, RT, 1.5 h, 70% (over 2 steps); g) Sodium-bis-[*N*-salicylidene-2-amino-isobutyrato]-cobaltate(III) (**20**), $PhSiH_3$, O_2, RT, 10 h; h) Ac_2O, pyr, RT, 99% (70% for **12** over 2 steps); i) **20**, Et_3SiH, O_2, RT, 14 h, 71%; j) TFDO, 0 °C, 2 h, 30%; k) $Zn(BH_4)_2$, RT, 1 h, 80% (1:1 d.r.); l) HCl, 0 °C (−41 °C for **4**); m) NaOH, RT, 12 h, 72% for **4** (over 2 steps) (70% for **3** (over 3 steps), 92% for **7**); n) $LiAlH_4$, −95 °C, 1 h; o) I_2, PIDA, *hν*, RT, 15 min, 30% of **16** from **14**; 34% of **15** and 15% of **16** from **13**; p) 40 mol% 4-NHAcTEMPO, PIDA, RT 3 h; q) $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, 2-methyl-2-butene, RT, 2.5 h, 73% (over 3 steps); r) $Al(OiPr)_3$, *i*PrOH, 100 °C, 1.5 h; s) Pd/C, H_2, RT, 12 h, 79% (over 3 steps); t) I_2, $Pb(OAc)_4$, *hν*, RT, 15 min; u) AgOAc, RT, 20 h, 50% (over 2 steps); v) $LiAlH_4$, 0 °C, 1 h, 74%. cod = 1,5-cyclooctadienene, TBAF = tetra-n-butylammonium fluoride, PIDA = phenyliodine(III) diacetate, TEMPO = 2,2,6,6-tetramethyl-piperidin-1-oxyl.

a methyl group (see the Supporting Information) showed that the C–H abstraction barrier at C16 is lowered by 1.6 kcal mol^{-1} by removing the inductively electron-withdrawing ester, thus ruling out a potential neighboring effect from the acetate group.

Derivative **14** was then reduced to the axial alcohol using $LiAlH_4$ and **3** was obtained after elimination and saponification. $Zn(BH_4)_2$ reduction of **14** afforded a 1:1 mixture of diastereomers which could be separated, and equatorial alcohol **4** was obtained with a similar sequence as that for **3**.

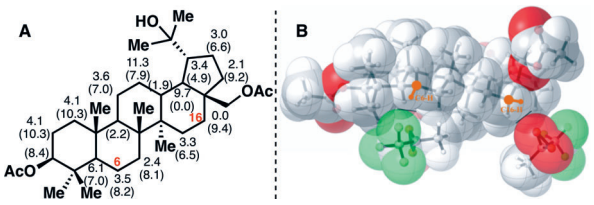


Figure 3. Analysis of site selectivities on **12** for TFDO. a) Relative activation enthalpies for equatorial C–H abstraction by DMDO ($\Delta\Delta H^{\ddagger}$) and relative stabilities of the free radicals ($\Delta\Delta E$, in parentheses) (kcal mol^{-1}). b) Juxtaposition of space-filling models of TFDO approaching C6–H and C16–H in **12**, to indicate the steric effects disfavoring C–H abstraction from C6–H. The relative orientations of TFDO and **12** were determined from the transition-state geometries shown in Figure S13 in the Supporting Information.

The structure of **4** has been attributed to the natural product heliantriol B2 in the literature. However, our NMR data did not perfectly match the reported data of this natural product,[19] which suggests a potential misassignment of heliantriol B2, since the structure of **4** was confirmed by X-ray crystallography. However, the lack of details in the isolation report might explain the observed discrepancies.

Functionalization of the A ring was then pursued using the C3 alcohol as a directing group. After benzylation of **2**, Hartwig's elegant 1,3-diol methodology was applied.[20] Silylation of the C3 alcohol, followed by C–H silylation at C23 and Tamao–Fleming oxidation afforded **11** in 24% yield (Figure 2 A). A two-step debenzylation delivered the natural product C23-hydroxybetulinic acid (**5**). Compound **5** has been previously synthesized from betulinic acid using a Baldwin-type C–H oxidation.[21] In that study, transformation of the alcohol into an oxime directing group, C–H oxidation with stoichiometric palladium, and then two-step regeneration of the alcohol was required. Hartwig's conditions avoided these four functional-group manipulation steps and do not necessitate protection of the C20–C21 alkene.

Functionalization of the C ring was achieved via the intermediacy of a hydroxyl radical at C20. Indeed, an oxygen radical could be generated using Suárez's conditions on substrate **12**,[22] resulting in C–H abstraction, iodine trap, and displacement leading to the formation of tetrahydropyran product **16** in 30% yield. While this type of transformation generally leads to tetrahydrofuran formation, the specific geometry of the system results in 1,6-hydrogen abstraction rather than 1,5-hydrogen abstraction. Compound **16** has been previously synthesized in a related transformation, but using lead tetraacetate in refluxing benzene for several hours.[23] Derivative **16** was then transformed into carboxylic acid **8** by removal of the acetate groups and a selective two-step oxidation of the primary alcohol. The tetrahydropyran ring in **16** proved to be very stable, and no hydrolysis conditions could be developed. Consequently, the venerable Barton nitrite ester reaction was considered to install a ketone at C12 in place of the cyclic ether.[24] Unfortunately, formation of the nitrite ester of the tertiary alcohol with NOCl in pyridine was ineffective, probably as a result of steric hindrance. The benzylic C–H oxidation directed by a peroxyl radical reported by both Kropf and Čeković then caught our attention.[25] To examine this directed oxidation, peroxide **13** was synthesized in two steps in a fashion very similar to that of **12**. Using triethylsilane instead of phenylsilane and removing the reductive work-up allowed the preparation of the desired peroxide. This substrate was then subjected to the same Suárez conditions and two products were isolated. The minor product was the previously isolated tetrahydropyran **16** (15% yield), whereas the major product was the desaturated compound **15** (34% yield). This compound corresponds to Čeković's findings, but interestingly, their exact conditions applied to **13** only afforded **16**. However, adding $Cu(OAc)_2$ to our reaction conditions enabled a slight improvement of the alkene yield, which is consistent with a mechanism involving hydrogen abstraction, followed by radical oxidation and proton elimination.[25f] Hydrolysis of the acetate groups delivered **7** which could serve as a platform for further modifications of the C ring.

A few reports have described oxidation of C13 using a hydroxyl radical at C28. The substrate for this transformation is the hydrogenated monoacetate compound **17**, and different authors reported the formation of the corresponding tetrahydrofuran product, albeit generally in low yields, after thermal or photoactivation of the hydroxyl radical. In our hands, however, this product could not be isolated despite the many reaction conditions that were tried. Instead, the formation of an iodinated product was observed when using lead tetraacetate and iodine under a sunlamp.[26] This product could not be purified and was subsequently treated with silver acetate in acetone.[22a] This two-step procedure initiated a rearrangement of the carbon skeleton, thus providing **18** in 50% yield (X-ray analysis), presumably by a 1,2-shift of the C13–C14 bond. From a medicinal chemist's point of view, this unexpected transformation allows the exploration of the bioactivity and physicochemical properties of a new family of compounds possessing an intriguing 6,6,5,7,5 pentacyclic structure. Finally, reduction of **18** with an excess of $LiAlH_4$ afforded triol **6**.

In parallel to these chemical studies, BMS screened several microorganisms to oxidize **2** (Figure 4). It was found that *Streptomyces fragilis* tranformed **2** into diol **9**, whereas *Bacillus megaterium* afforded diol **10** instead.[27]

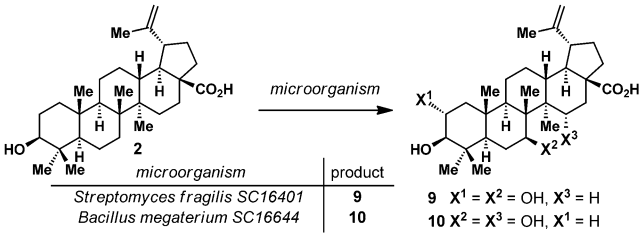
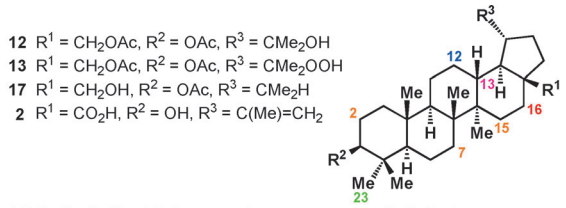


Figure 4. Microbial oxidations of betulinic acid (**2**).

The panel of aliphatic C–H oxidations that were developed on the lupane core are summarized in Figure 5. Alcohol **12** can serve both for the directed oxidation of C12 and nondirected oxidation of C16 with TFDO (entries 1, 2, and 4). No other oxidants were found to enable the same reaction



Entry	Conditions	12	13	17	2
1	PIDA, I_2, $h\upsilon$	C12	C12	decomp.	–
2	$Pb(OAc)_4$, I_2, $h\upsilon$	C12	trace	C13	–
3	NOCl, pyr	N.R.	–	–	–
4	TFDO	C16	–	–	–
5	DMDO	N.R.	–	–	–
6	$KMnO_4$	N.R.	–	–	–
7	CrO_3, $^nBu_4NIO_4$[28a]	decomp.	–	–	–
8	$RuCl_3 \cdot xH_2O$, $KBrO_3$[28b]	N.R.	–	–	–
9	Crabtree's Ir catalyst[28c]	N.R.	–	–	–
10	Hartwig's 1,3-diol	–	–	–	C23[a]
11	*Streptomyces fragilis*	–	–	–	C2/C7
12	*Bacillus megaterium*	–	–	–	C7/C15

Figure 5. Summary of aliphatic C–H oxidation panel used on the lupane skeleton. [a] $R^1 = CO_2Bn$.

(entry 5–9).[28] From hydroperoxide **13** arose alkene **15** and ether **16**, two compounds oxidized at C12 (entry 1). Alcohol **17** is the precursor of the C13 oxidation followed by skeletal rearrangement (entry 2) and benzylated **2** is that of C23 oxidation (entry 10). Interestingly, all the developed enzymatic C–H oxidations are orthogonal to the chemical C–H oxidations (entry 11 and 12), which illustrates the complementarity of these two approaches.

The solubility of these new oxidized compounds was tested in simulated intestinal fluids and compared to reference compounds (**1** or **2**) possessing the same oxidation state at C28 (Table 1). The solubility was measured in both fasted state (assay 1) and fed state (assay 2).[29] The solubility of **3** and **7** in assay 1 is over 100-fold higher than that of **1**, while nearly identical in assay 2. The solubility of **8** is 17-fold higher in assay 2 than that of **2**, albeit somewhat lower in assay 1. Consequently, adding one hydroxy group to **1** and **2** drastically improved their solubility. This work clearly demonstrates the unpredictable nature of skeletal oxidation on solubility since **4** has almost identical solubility to **1** despite having one more alcohol, and diols **9** and **10** are less soluble than **2** (only a slight improvement seen in assay 2). Finally, the solubility of the rearranged product **6** is the lowest of all the family.

Table 1: Relative solubility enhancement of the oxidized compounds.

Entry	Substrate	R^1	Relative Solubility Enhancement: Assay 1 (FaSSIF)[a]	Relative Solubility Enhancement: Assay 2 (FeSSIF)[b]
1	**3**	CH_2OH	274×	no change
2	**4**	CH_2OH	8.00×	0.077×
3	**7**	CH_2OH	121×	0.357×
4	**6**	CH_2OH	no change	0.077×
5	**5**	CO_2H	0.056×[c]	0.115×[c]
6	**8**	CO_2H	0.112×[c]	17.4×[c]
7	**9**	CO_2H	0.019×[c]	3.38×[c]
8	**10**	CO_2H	0.002×[c]	0.462×[c]

[a] Solubility ratio substrate/**1** in the fasted state simulated intestinal fluid. [b] Solubility ratio substrate/**1** in the fed state simulated intestinal fluid. [c] Solubility ratio substrate/**2**. R^1 refers to the position shown in the structure of Figure 5 (C17).

To the best of our knowledge, this is the first published systematic study using both chemical and enzymatic methods for aliphatic C–H oxidation with the goal of improving physical properties. It is our hope that this study will help to alleviate some of the empiricism in the process of screening oxidants and that the combination of spectroscopy, crystallography, and computation along with a systematic C–H oxidation panel will serve to accelerate future efforts. C–H oxidation clearly merits consideration by medicinal chemists confronted with the challenge of an exciting chemotype whose further development is hampered by extreme insolubility.

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