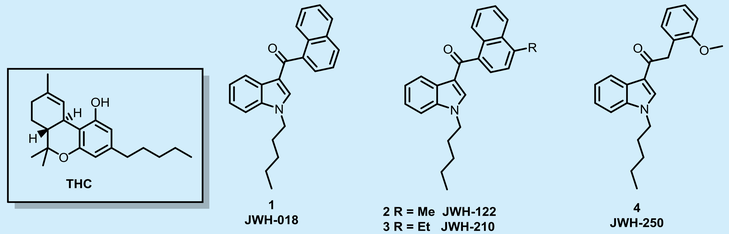
Regioselective Friedel-Crafts Type Acylation of Indoles: An Improvement on the Synthesis of Cannabimimetic Indole Metabolites

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Introduction

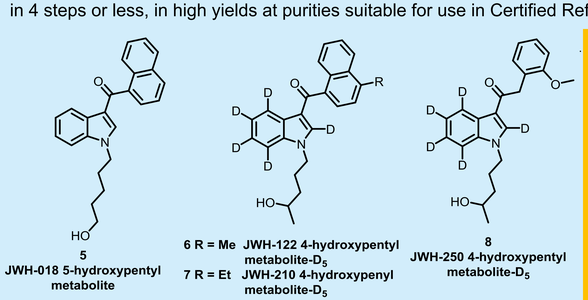
Indole cannabimimetics, commonly referred to as “Spice,” are psychoactive designer drugs created to mimic the effects of cannabis. While the chemical structures of these compounds do not resemble that of Δ^9-tetrahydrocannabinol (THC), the active component of cannabis, they show high binding affinities for the CB_1 and CB_2 cannabinoid receptors. In the early 2000s, Dr. John Huffman identified different structural subclasses of these cannabinoids that possessed very high binding affinities for the cannabinoid receptors through extensive structure-activity relationship studies. In particular, he discovered that naphthoylindoles (JWH-018 **1**, JWH-122 **2** and JWH-210 **3**) and phenylacetylindoles (JWH-250 **4**) are highly active in the endocannabinoid system of mammals.[1,2,3]



Illegal use and abuse of these synthetic cannabinoids has created a need for certified reference standards to identify and quantify these drugs in clinical and forensic samples. Additionally, due to their fast metabolism *in vivo*[4], the preparation of the major metabolites and their stable-isotope labeled internal standards is of high priority. To this end, a regioselective, highly efficient synthesis of 4-hydroxypentyl or 5-hydroxypentyl metabolites of JWH-018, JWH-210, JWH-250 and JWH-122 has been completed for the development of analytical reference and internal standards.

Synthetic Design

Synthesis of the 4-hydroxypentyl or 5-hydroxypentyl metabolites of JWH-018, JWH-210, JWH-250 and JWH-122 can be completed in a 3-4 steps, however in our hands, the key acylation step has been problematic and low yielding despite many published methods.[1,2,3] Our goal was to optimize the yield with the intent of applying it to deuterium labeled substrates which are much more costly to produce. A tin-mediated Friedel-Crafts type regioselective acylation of indole/indole-D_7 dramatically increased the overall yields of these molecules. This method worked well with free indole, eliminating the need for N-protection to give a shorter overall synthesis. JWH metabolites **5-8** were synthesized regioselectively in 4 steps or less, in high yields at purities suitable for use in Certified Reference Standards.



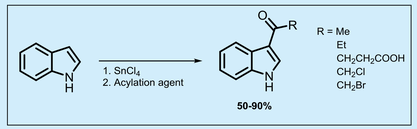
INTERNAL STANDARD DESIGN

- **Most common stable labels: D, ^{13}C, ^{15}N**
- **Placement of labels determined by end-use (i.e. LC/MS or GC/MS) and fragmentation patterns.**
- **Major fragment ions must retain label to be a candidate for an internal standard.**

References

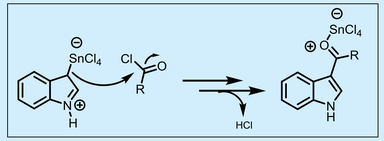
1. Bioorg. Med. Chem., **2005**, 13, 89;
2. Bioorg. Med. Chem. Lett., **2005**, 15, 4110;
3. Bioorg. Med. Chem., **2012**, 20, 2067;
4. Front. Behav. Neurosci., **2011**, 5, 60;
5. Org. Lett., **2001**, 3, 1005-1007.

Sn-Mediated Regioselective Acylations of Indole

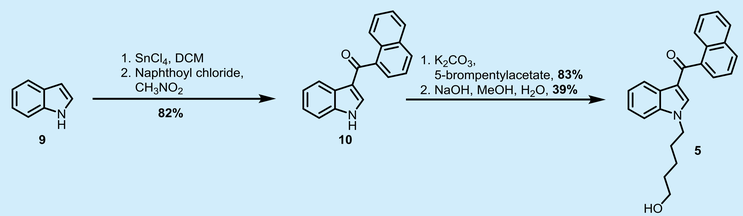


In 2001, Ottoni et. al. published Sn-mediated acylations of indole using small molecule acid chlorides and anhydrides.[5] However, there was no application to bulkier aroyl, naphthoyl or phenylacetyl chlorides. Based on the mechanism of the reaction, we decided to apply this method to bulkier substituents.

The Sn-mediated acylation of indole with naphthoyl and phenylacetyl chlorides, gave much better results compared to previous Et_2AlCl methods increasing yields by as much as 8-fold. When $SnCl_4$ is added to indole, an addition complex is formed, causing a swift color change to deep royal blue. Upon addition of the acid chloride, a deep red color forms and persists. The reaction goes to completion within 1 hr, with no starting indole/indole-D_7. When applied to isotopically labeled substrates, no isotope effects were seen, giving similar reaction times and yields. In addition, no scrambling or loss of deuterium was observed.

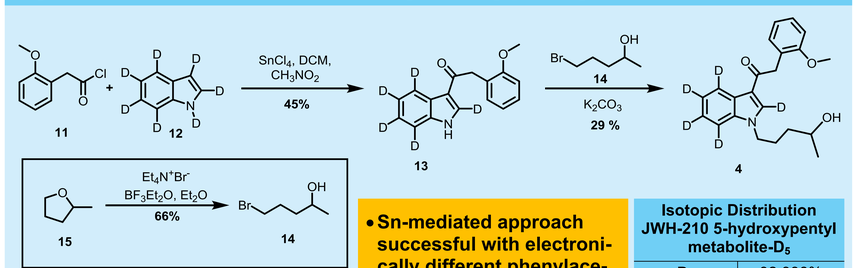


Synthesis of native JWH-018 5-hydroxypentyl metabolite



$SnCl_4$ acylation yield was 82% vs. 20% using Et_2AlCl and 10% using MeMgBr

Synthesis of JWH-250 5-hydroxypentyl metabolite D_5

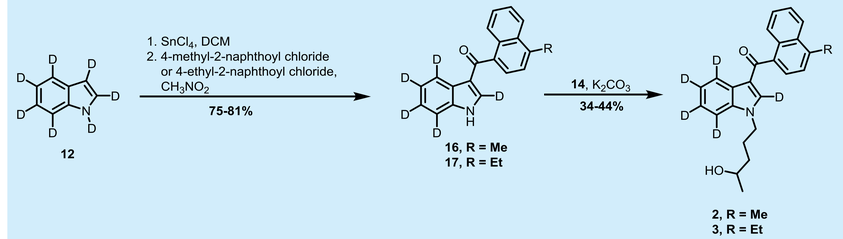


- **2-Methoxyphenylacetyl chloride prepared from corresponding carboxylic acid using thionyl chloride.**
- **N-alkylation achieved without the need for an OH protecting group.**

- **Sn-mediated approach successful with electronically different phenylacetyl chloride - 45 % yield vs 14% yield using Et_2AlCl.**
- **High isotopic purity retained throughout the reaction sequence.**

Isotopic Distribution JWH-210 5-hydroxypentyl metabolite-D_5	
D_5	92.338%
D_4	7.408%
D_3	0.254%
D_2	0.000%
D_1	0.000%
D_0	0.000%

Synthesis of JWH-122 and JWH-210 5-hydroxypentyl metabolites-D_5



- **4-Ethyl-2-naphthoyl chloride prepared from corresponding carboxylic acid using thionyl chloride.**
- **No scrambling or loss of deuterium observed throughout reaction sequence.**
- **Additional substituents on the naphthoyl ring made no impact on reactivity or yield.**

Isotopic Distribution of JWH-210 5-hydroxypentyl metabolite-D_5	
D_5	92.530%
D_4	7.247%
D_3	0.184%
D_2	0.040%
D_1	0.000%
D_0	0.000%

Isotopic Distribution of JWH-122 5-hydroxypentyl metabolite-D_5	
D_5	92.670%
D_4	7.059%
D_3	0.212%
D_2	0.052%
D_1	0.007%
D_0	0.000%

Results & Discussion

- Naphthoyl chlorides or phenylacetyl chlorides that were not commercially available, were prepared from their respective carboxylic acids using thionyl chloride.
- Sn-mediated Friedel-Crafts type acylation of indole (**9**) or indole-D_7 (**12**) with naphthoyl and phenylacetyl chlorides gave dramatically increased yields of 45-82%.
- Electronic differences between phenylacetyl chloride **11** and the naphthoyl chlorides afforded different reactivity, and a slightly lower yield was obtained when **11** was used. When using Et_2AlCl on native indole as the acylating agent, only a 14% yield was obtained compared to 45% yield with the new method.
- Final N-alkylation was achieved using 5-bromopentylacetate or **14** under mild conditions to yield the final synthetic cannabinoid metabolites. In the case of JWH-018 5-hydroxypentyl metabolite, hydrolysis of the remaining ester afforded the final metabolite.
- Literature methods used in our labs to prepare the synthetic cannabinoids (Et_2AlCl and MeMgBr), provided 10-20% yields at best.

Conclusion

- Sn-mediated Friedel-Crafts type acylations of indole/indole-D_7 provides an efficient, mild and high yielding route to synthetic cannabinoids.
- This new method significantly increases the yield not only of the key step in this reaction sequence, but overall yields of the final spice cannabinoid metabolites, when compared to methods using Et_2AlCl or MeMgBr as the acylating agents.
- All metabolites were prepared in ≥ 98% chromatographic purities and deuterium labeled products retained high isotopic purities.
- All metabolites synthesized were developed into Certified Reference Materials suitable for quantitation for LC/MS or GC/MS testing applications in clinical toxicology, forensic analysis or urine drug testing.