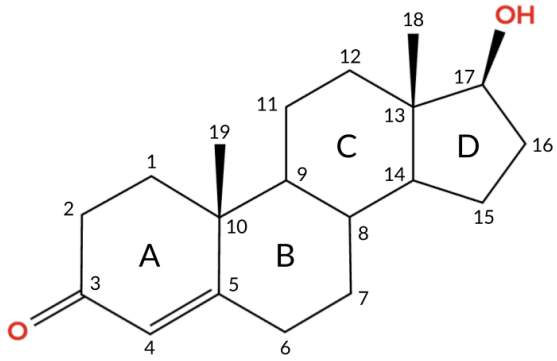
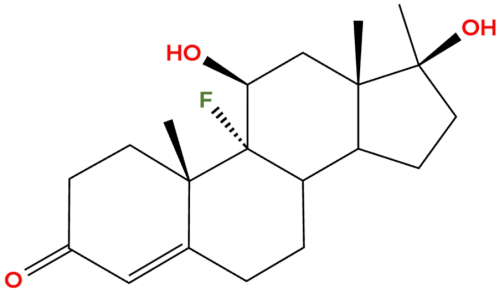
Explained: Anabolic Steroids

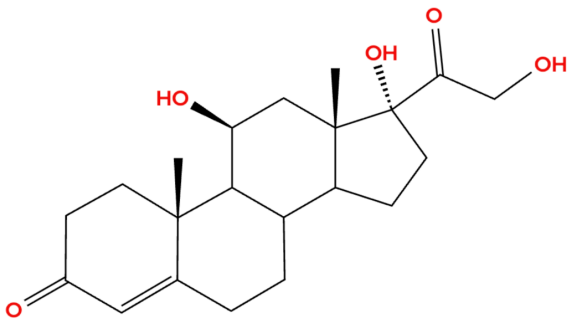
What are Anabolic Steroids



Anabolic steroids are synthetic drugs chemically related to testosterone, which is the primary male sex hormone. They mimic testosterone's effect by binding to androgen receptors in the body, stimulating muscle tissue growth (anabolic effects), and leading to the development of male sex characteristics (androgenic effects). Anabolic steroids all share a similar structure of having four fused rings, three 6-membered rings and one five-membered ring. Above is testosterone with each carbon and each ring labeled, but different anabolic steroids have structural modifications altering their potency, bioavailability, and side effects.



Halotestin



Cortisol

Anabolic steroids can have both anti-catabolic and anabolic effects. Cortisol, for example, is a glucocorticoid that is produced by the adrenal glands. When cortisol levels are high, cortisol binds to glucocorticoid receptors and induces the catabolism of muscle proteins. However, many anabolic steroids, such as halotestin (also known as fluoxymesterone), can competitively bind to the active site (ligand binding pocket) of glucocorticoid receptors and displace cortisol. This results

in an anti-catabolic effect, since cortisol binds less to glucocorticoid receptors in the presence of halotestin and can no longer induce muscle protein degradation.

Halotestin results in competitive inhibition, so high enough concentrations of halotestin mean that cortisol activity in glucocorticoid receptors can be almost completely suppressed. This is because halotestin can displace cortisol and saturate all of the glucocorticoid receptors if the concentration of halotestin is very high, meaning that cortisol cannot bind to any appreciable extent. So, certain anabolic steroids like halotestin can have anti-catabolic effects that can vary based on the dosage, since it is possible to control how much the glucocorticoid receptors are saturated by controlling the amount of the competitive inhibitor. Interestingly enough, halotestin can also have anabolic effects by binding to androgen receptors and inducing muscle growth. This makes halotestin extremely potent because it exhibits both anti-catabolic and anabolic effects.

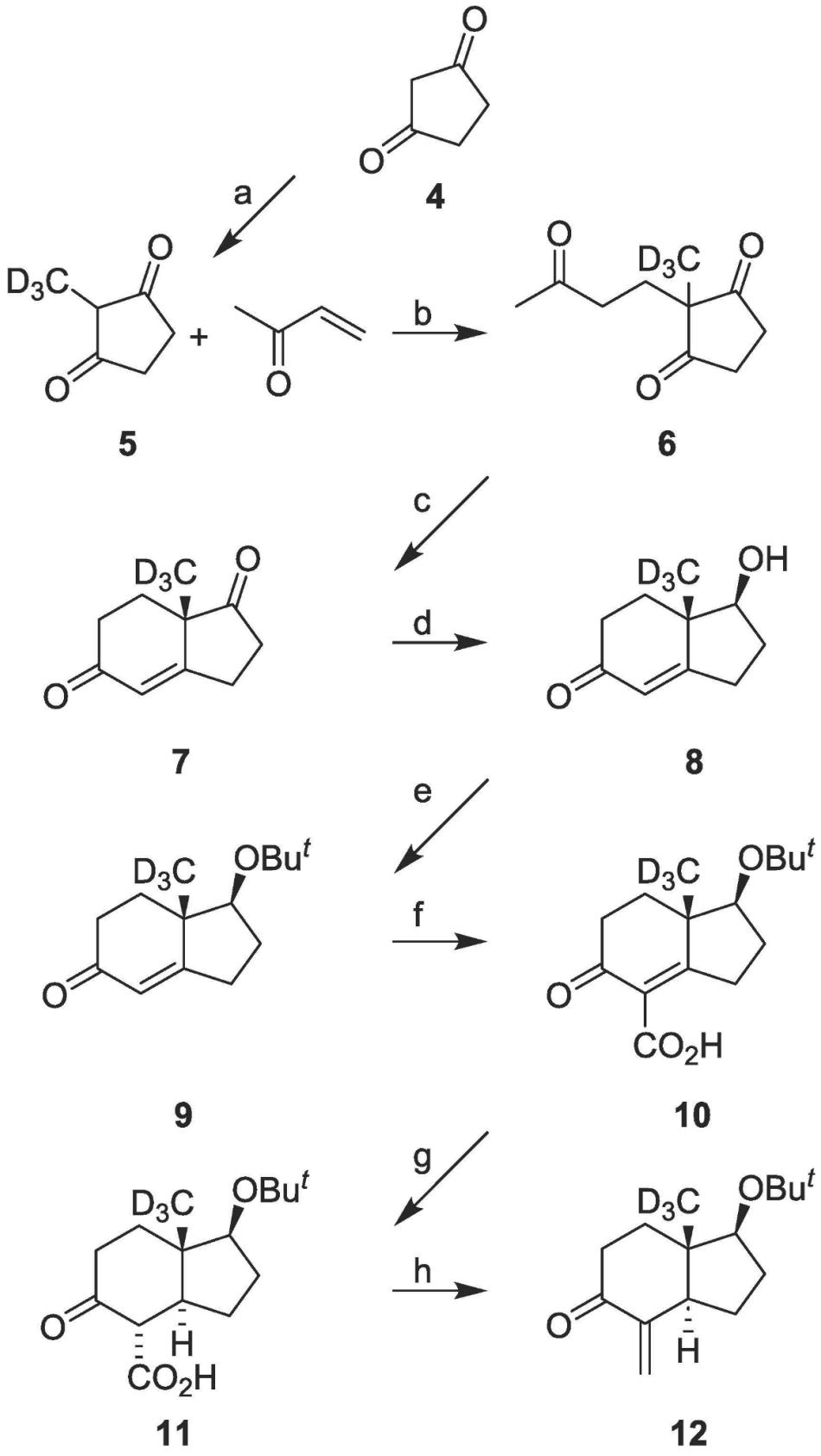
How are Anabolic Steroids Made

Anabolic steroids are synthesized either through total synthesis methods from non-steroid precursors or semisynthetic modifications from steroid-like precursors.

Total Synthesis

There are many different and very elaborate methods of synthesizing anabolic steroids from non-steroid precursors. Historical examples include Bechmann's synthesis (1940) and the Torgov Synthesis (1950) which construct the 4 rings sequentially. Modern routes have evolved to use catalysts and require less steps to improve yields.

This paper from Mingxing Qian and Douglas F. Covey details the total synthesis of testosterone starting with 1-3-cyclopentanedione.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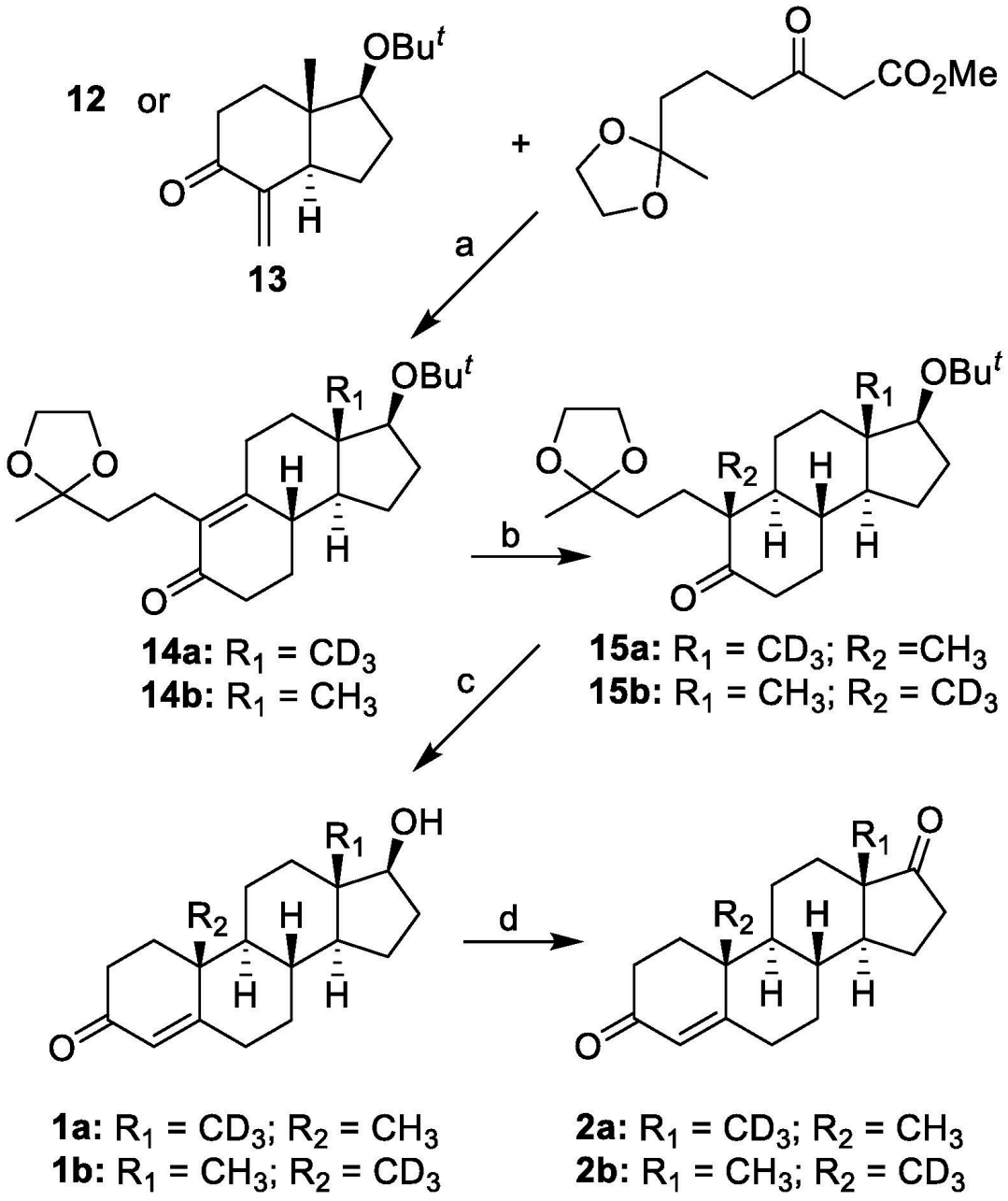
Scheme 1. Reagents: (a) 0 °C, 1 h then 65 °C, 72 h (36 %); (b) H2O/AcOH, methyl vinyl ketone, under N2, 70 °C, 3 h (91 %); (c) l-proline, DMF, under N2, 15 °C, 72 h followed by aqueous H2SO4, 95 °C, 3 h (66 %); (d) NaBH4, EtOH, −5 to 5 °C, 1 h (99 %); (e) H3PO4, BF3·Et2O, isobutylene, −78 °C then −5 °C, 6 h and 23 °C, 12 h (70 %); (f) methyl magnesium carbonate, DMF, 120 °C, 3 h (91 % crude); (g) Pd/BaSO4, MeOH, 23 °C, H2, (55 psi) (~100 % crude); (h) aqueous HCHO, DMSO, piperidine, 0 °C, 2 h (98 % crude).



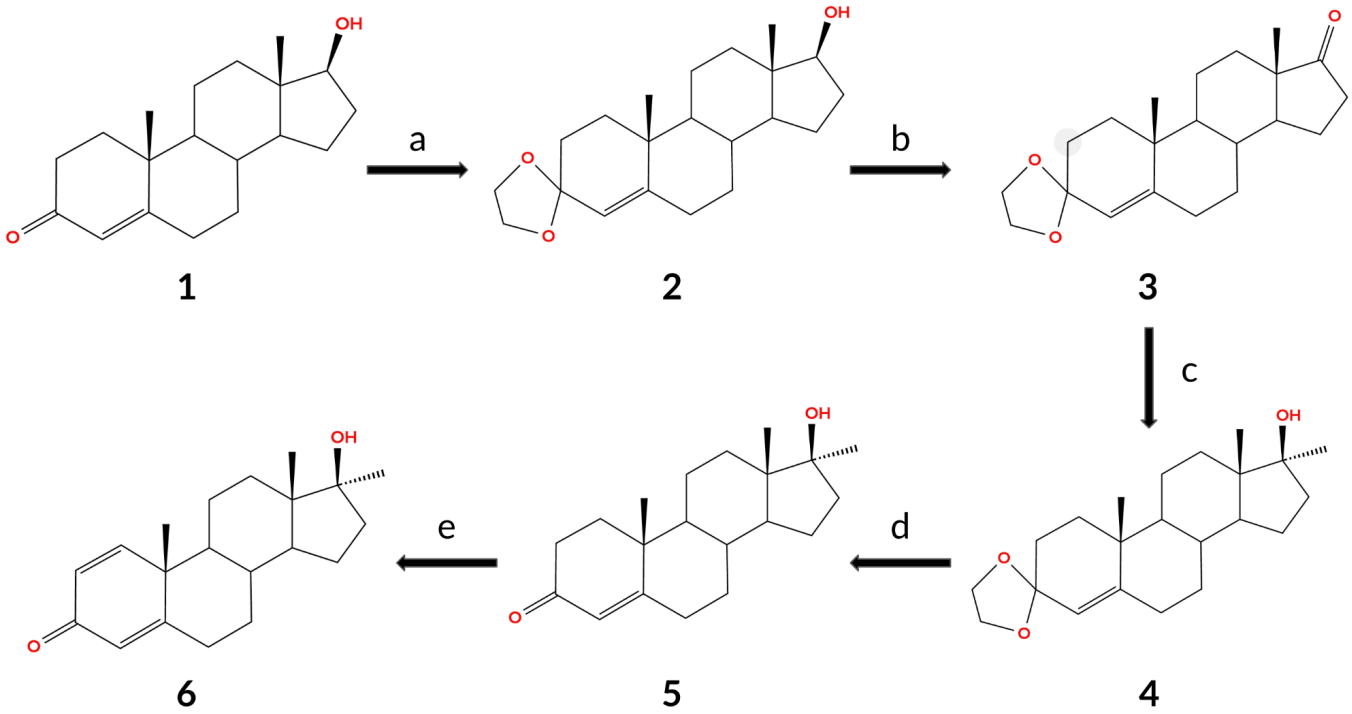
Scheme 2. Reagents: (a) i: NaOMe, MeOH, 23 °C, 16 h; ii: 5 N NaOH, 1 h; iii: 6 N HCl, 80 °C, 2 h (14a, 77 % crude from 12; 14b, 39 % crude from 13); (b) anhyd·NH3, THF, −78 °C,1 h, then CD3I or

CH3I addition (15a, 21 % from crude 14a); 15b, (57 % from crude 14b); (c) 3 N HCl, MeOH, reflux, 24 h (1a, 61 %; 1b, 86 %); (d) NaHCO3, Dess-Martin periodinane, CH2Cl2, 23 °C, 2 h (2a, 96 %; 2b, 97 %).

Product C is testosterone and product D is androstenedione, both of which can be further modifications to synthesize more complex steroids. Total synthesis methods have very low yields with the one above having a yield of ~3% after 11 steps. Semi-synthetic pathways starting with naturally produced steroid analogs are more desirable.

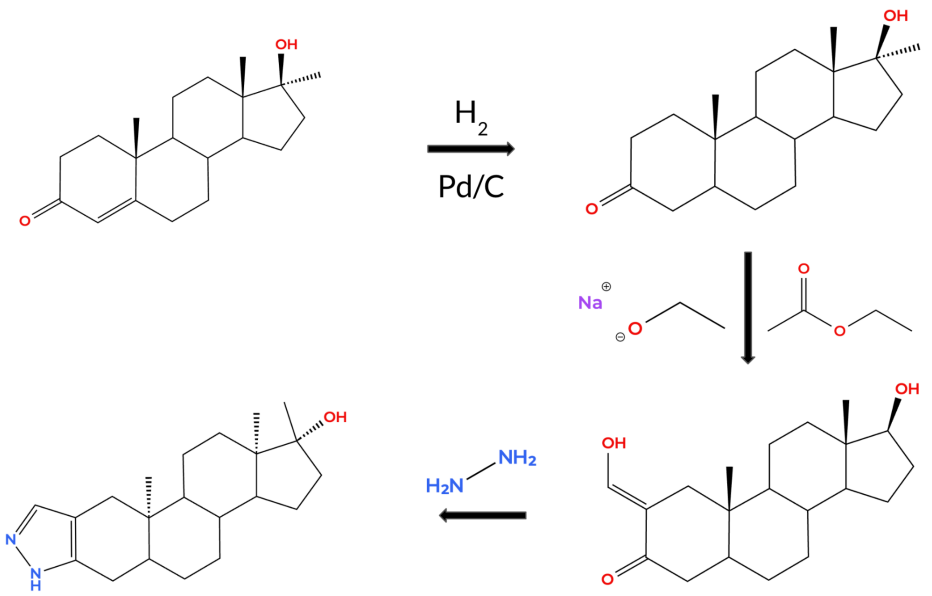
Synthetic Steroid Modifications

Testosterone or androstenedione are a common precursor for steroid modifications. For example, testosterone could be converted to dianabol through the pathway. This is useful because dianabol is an oral steroid so it doesn't need to be injected and has greater anabolic properties while being less androgenic. The carbonyl in 1 can be protected through an acetal protecting group (yielding 2) so the 17a hydroxyl group can be oxidized into a ketone (3). From there a methyl Grignard reagent can produce methyl testosterone (4). The carbonyl can be deprotected to yield product 5 and SeO_2 can selectively oxidize the C1-C2 bond producing dianabol as the final product.



Another modification is adding a pyrazol ring yielding winstrol (stanizol) as a product. This modification drastically reduces its androgenic profile while maintaining its anabolic properties and making it more metabolically stable. This is done using 5 as a precursor and reducing the double bond between C4-C5. An Aldol condensation reaction can be performed next using ethyl

formate and sodium ethoxide. From there, the intermediate can be reacted with hydrazine under acidic conditions to form the product.



How do Anabolic Steroids Enter the Body

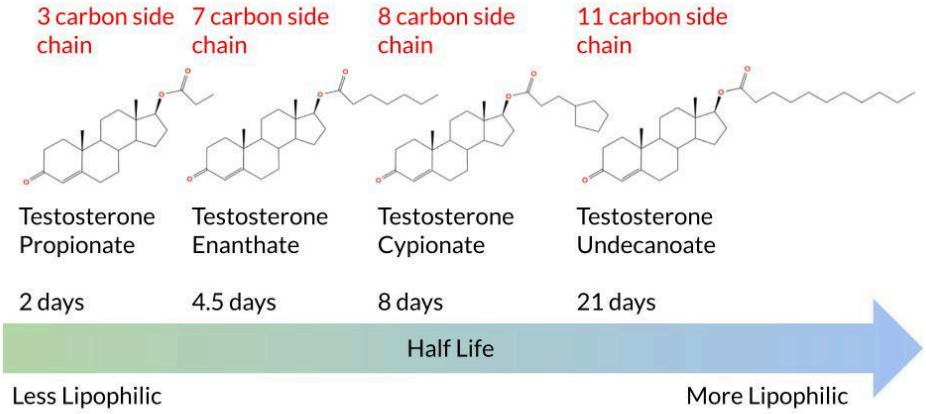
There are two primary pathways of taking anabolic steroids—by injecting them or taking them orally.

Injection:

The steroids are dissolved in vegetable oils like arachis oil along with benzoyl benozoate or benzyl alcohol for bacteriostatic properties. Steroids are nonpolar so they dissolve in oil, which is a nonpolar solvent. When injected, an oil deposit forms in the muscle tissue and then gradually releases into the interstitial fluid, which is the fluid between cells in tissues and the bloodstream.

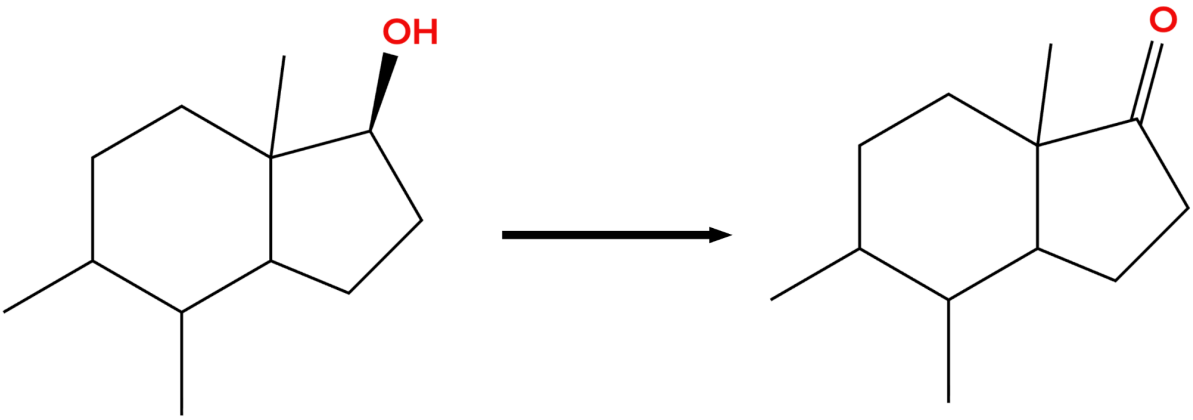
Since the oil deposit is nonpolar, the steroid molecule will begin within the oil deposit and diffuse out into the aqueous surroundings over time. Testosterone has a half-life of 10 minutes in the body, which is too short to have a substantial anabolic effect so steroids are esterified to last longer inside the body. The ester will have a hydrocarbon chain and the longer the hydrocarbon chain, the more gradual it will metabolize. This is for two reasons. The first is that it is more lipophilic, so it will stay within the oil deposit longer and not enter the aqueous phase where it will be metabolized by esterase. Esterase hydrolyzes the ester bond, so more nonpolar esters will be harder for esterase to access the ester bond and coordinate water. Additionally longer chains are

less sterically accessible for esterase to metabolize. Testosterone has a half-life of 10 minutes if injected by itself but the longer the ester chain, the more lipophilic and the longer it will stay in your system.

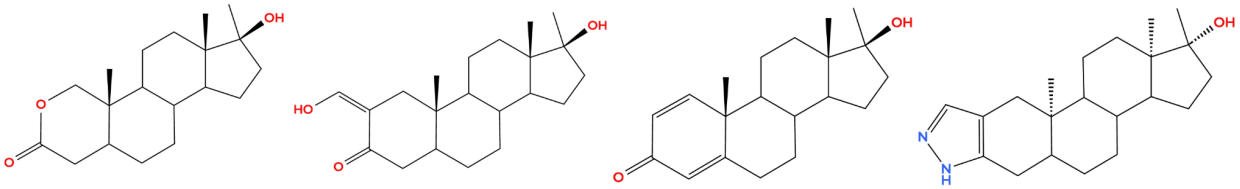


Oral Delivery

Oral steroids are taken in capsules where they are rapidly absorbed in the gastrointestinal tract within 1-2 hours. Most steroids have poor oral bioavailability, so if 25 mg of testosterone is taken then only 1 mg (4%) would actually make its way into the bloodstream. Liver enzymes metabolize steroids by performing modifications to make them more water soluble so they can be excreted into urine. This happens through two phases, aptly named phase 1 and phase 2. Phase 1 adds and reveals functional groups which become targets for phase 2 reactions. In phase two, enzymes perform conjugation reactions to inactivate the molecule and make it significantly more polar and water soluble to be excreted into urine. Phase 1 includes metabolic steps like A ring reduction and 17-β oxidation which is shown below.



The primary approach to enable steroid bioavailability is through 17-α methylation. This prevents 17-β oxidation and all common oral anabolic steroids have this modification.



Anavar Anadrol Dianabol Winstrol

The liver enzyme cytochrome p450s (CPY450) is sterically hindered by the addition of a methyl group. Because of this, the steroids are able to enter the bloodstream in greater amounts. Orally active steroids have greater hepatotoxicity (liver toxicity) due to the liver needing to work harder to metabolize them. This causes inflammation and cell damage and long-term steroid use can lead to the development of liver tumors, cardiovascular disease, and infertility.

Binding to Androgen Receptors

Steroids bind the androgen receptors. There are androgen receptors all throughout the body, but they are broadly broken down into two categories. There are androgen receptors that activate male sex characteristics in the voicebox, skin, hair follicles, and prostate. Anabolic steroids that bind strongly to these are described as more androgenic. There are also androgen receptors in the muscles and bone and steroids that bind more strongly to these androgen receptors are more anabolic. In other words, androgenic means it will activate male sex characteristics and anabolic means they will promote muscle growth. Typically, people want to minimize the androgenic effects and maximize the anabolic effects.

This depends on the androgen receptor. There are 4 parts of the androgen receptor. The first is the ligand binding domain (LBD) where the anabolic steroid binds to. The next is the hinge which connects the LBD to the DNA binding domain or DBD. In the androgen receptor family of proteins, this region is the most conserved. Finally, there is the N-terminal domain which binds other cofactors to regulate gene expression.

The process works by the steroid binding to the hydrophobic pocket in the LBD. The 3-ketone group forms hydrogen bonds with LBD amino acids Thr877 and Gln711 in addition to the 17-β hydroxyl group also forming hydrogen bonds. The rest of the binding is due to van der Waals forces. Then the androgen receptor moves into the nucleus where it can bind to the DNA so genes can be expressed involved in muscle growth, protein synthesis, and other anabolic processes.

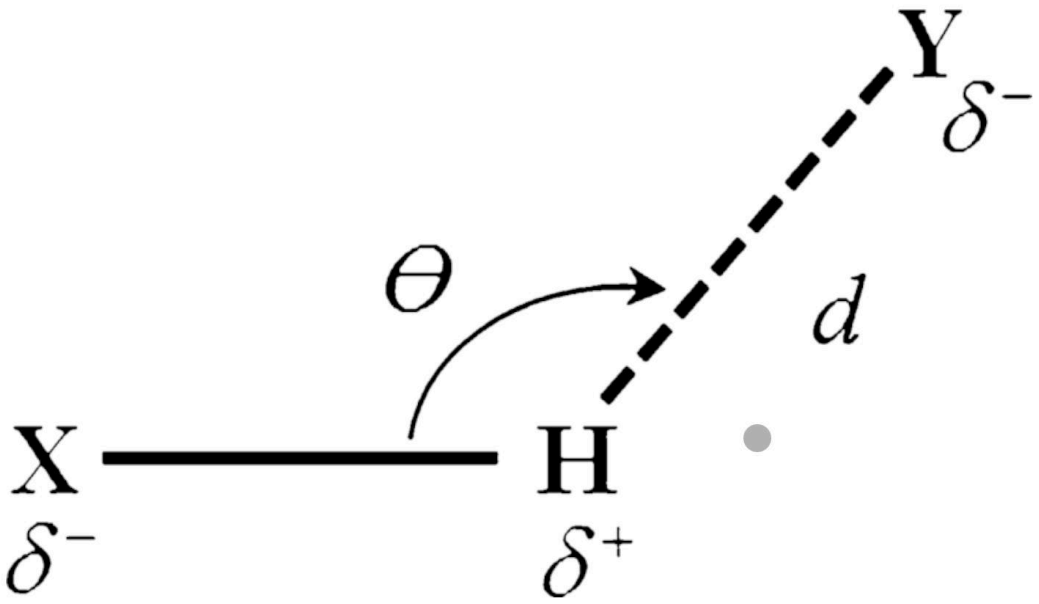
The anabolic effects of anabolic steroids is dictated by how strong it can bind to androgen receptors within muscle and bones. One strategy to change the amount of cc double bonds in the

rings which make the steroid flatter. Trenbolone or tren has C9-C10, C11-C12, C1-C2 double bonds and binds very strongly to receptors. One rationalization for the stronger binding of flatter steroids to the LBD is the increased hydrogen bonding capacity of the 17-β hydroxyl group. In flatter steroids like DHT and THG, there is a shorter distance between the 17-β hydroxyl group and a key Asn705 residue, which results in a shorter—and thus stronger—hydrogen bond. As well as this, the 17-β hydroxyl group is able to position itself at an angle closer to 180 degrees compared to testosterone. A 180 degree angle is the ideal angle for hydrogen bonding because this results in the best overlap of the hydrogen bond acceptor (Asn705) and the hydrogen bond donor (17-β hydroxyl group). So, it is both the shorter hydrogen bond length and the more linear angle that optimizes the binding of flatter steroids. Originally, tren was developed for horses and cattle but bodybuilders use it for its immense anabolic effects. Another method is to add methyl groups at various carbon centers to change the structure and binding affinity of the steroid to the androgen receptor. The steroid Halotestin has the addition of fluorine at the 9a position which allows for extremely strong hydrogen bonding.

Table 3. *H-bonding angles (°) and distances (Å) calculated from modeling results*

	Residue			
Ligand	Gln_{711} $H_{Ne2,Gln711}$–$O_{3,lig}$	Arg_{752} $H_{Nη2,Arg752}$–$O_{3,lig}$	Asn_{705} $O_{δ1,Asn705}$–$H_{O17,lig}$	Thr_{877} $O_{γ1,Thr877}$–$H_{O17,lig}$
Testo (1)	144.3° (2.56 Å)	104.6° (2.84 Å)	149.2° (2.01 Å)	151.9° (1.90 Å)
Testo (2)	—	104.5° (2.92 Å)	139.9° (2.04 Å)	144.7° (2.02 Å)
DHT	—	121.1° (2.36 Å)	147.7° (1.90 Å)	148.1° (1.89 Å)
THG (1)	136.7° (2.91 Å)	103.3° (2.72 Å)	170.8° (1.90 Å)	144.7° (1.90 Å)
THG (2)	—	100.4° (2.67 Å)	163.4° (1.99 Å)	146.4° (1.86 Å)

(1) Complex with Gln 711 in the vicinity of the ligand; (2) complex with Gln 711 farther from the ligand.



It is worth noting that modifications to the 17-β hydroxyl group can decrease the affinity of the steroid to the LBD of androgen receptors. For example, 17-β esterification like in testosterone undecanoate completely destroys hydrogen bonding between the 17-β hydroxyl group and the

Asn705 residue of the androgen receptor. How, then, might this esterification be beneficial to increasing steroid potency if it disallows binding to the androgen receptor? This is because the esterified steroid is a prodrug—the only purpose of the ester group is to bypass first-pass metabolism. Later on, esterase cleaves the ester group to release the active anabolic steroid. Thus, the esterified 17-β hydroxyl group is designed as a protecting group that can increase bioavailability, just like how prodrugs are inactive forms of a drug that are later metabolized to release the active form of the drug.

Significance

Legitimate Uses

The main use of anabolic steroids is testosterone replacement therapy (TRT) for aging men. 13 million men in the US over age 45 suffer from low testosterone. However, only a small fraction of these men actually receive TRT to get their testosterone levels in the normal ranges. Another use of testosterone is to treat muscle wasting. Many chemotherapy treatments are taxing on the body and patients will lose a significant amount of muscle mass so anabolic steroids are often recommended by doctors to assist in recovery. HIV/AIDS patients, burn victims, and patients post surgery also benefit. Testosterone also plays a role in treating the delayed onset of puberty in boys.

Illicit Uses

Steroids are often abused especially in the fitness space. Many bodybuilders take steroids to compete in bodybuilding shows and gain significantly more muscle than is necessary. Typically, illicit users take doses of anabolic steroids on the order of 10 to 100 times higher than legitimate medical uses. Many young men also take anabolic steroids to increase lean muscle mass and reduce body fat, which is done primarily for aesthetic purposes. While steroids themselves aren't addictive, people get addicted to the way they look and they way the feel while on them. Users are supposed to cycle steroids and take them for 8-12 weeks and take a month off to allow their body to recover and regain the ability to naturally produce testosterone. However, this often doesn't happen and people keep taking steroids without cycling off, leading to the body becoming unable to produce testosterone on its own ever again.