

- (54) **FITNESS ASSAY AND ASSOCIATED METHODS**
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- (\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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**C12Q 1/70** (2006.01)
- (52) **U.S. Cl.** ..... **435/5**
- (58) **Field of Classification Search** ..... **435/5;**  
514/357, 332, 478, 482, 228.2
- See application file for complete search history.

(57) **ABSTRACT**

The present invention provides an assay for determining the biochemical fitness of a biochemical species in a mutant replicating biological entity relative to its predecessor. The present invention further provides a continuous fluorogenic assay for measuring the anti-HIV protease activity of protease inhibitor. The present invention also provides a method of administering a therapeutic compound that reduces the chances of the emergence of drug resistance in therapy. The present invention also provides a compound of formula (I) or a pharmaceutically acceptable salt, a prodrug, a composition, or an ester thereof, wherein A is a group of formulas (A), (B), (C) or (D); R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>5</sup> or R<sup>6</sup> is H, or an optionally substituted and/or heteroatom-bearing alkyl, alkenyl, alkynyl, or cyclic group; Y and/or Z are CH<sub>2</sub>, O, S, SO, SO<sub>2</sub>, amino, amides, carbamates, ureas, or thiocarbonyl derivatives thereof, optionally substituted with an alkyl, alkenyl, or alkynyl group; n is from 1 to 5; X is a bond, an optionally substituted methylene or ethylene, an amino, O or S; Q is C(O), C(S), or SO<sub>2</sub>; m is from 0 to 6; R<sup>4</sup> is OH, =O (keto), NH<sub>2</sub>, or alkylamino, including esters, amides, and salts thereof; and W is C(O), C(S), S(O), or SO<sub>2</sub>. Optionally, R<sup>5</sup> and R<sup>6</sup>, together with the N—W bond of formula (I), comprises a macrocyclic ring.

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**9 Claims, 5 Drawing Sheets**

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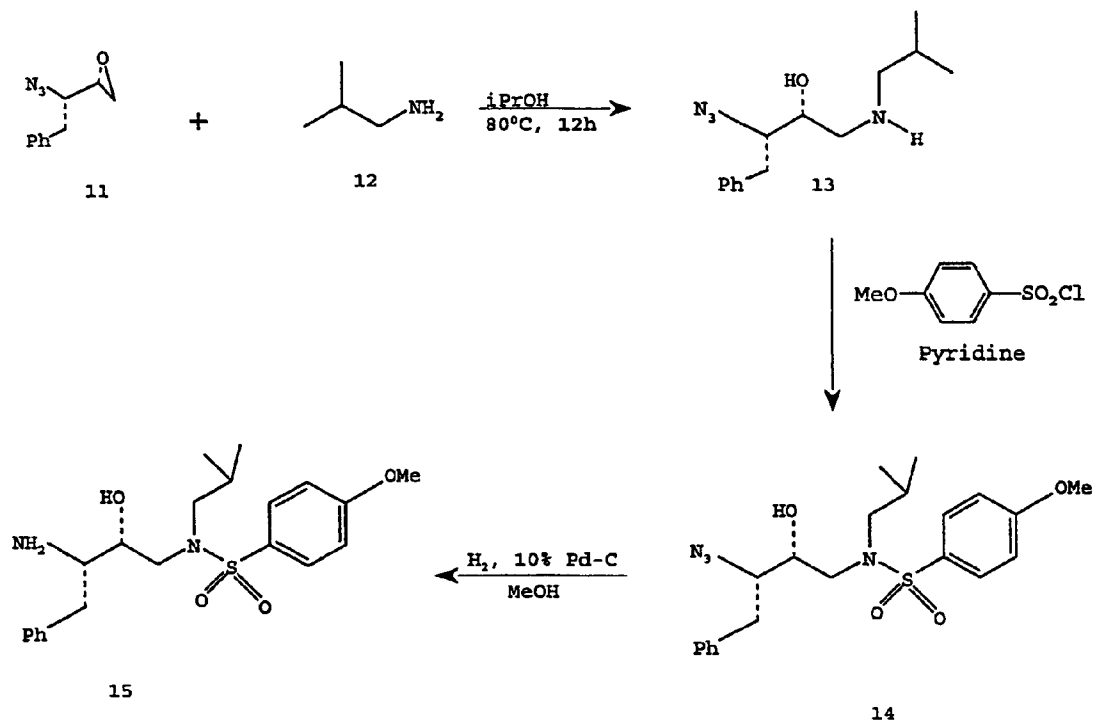


Fig. 1

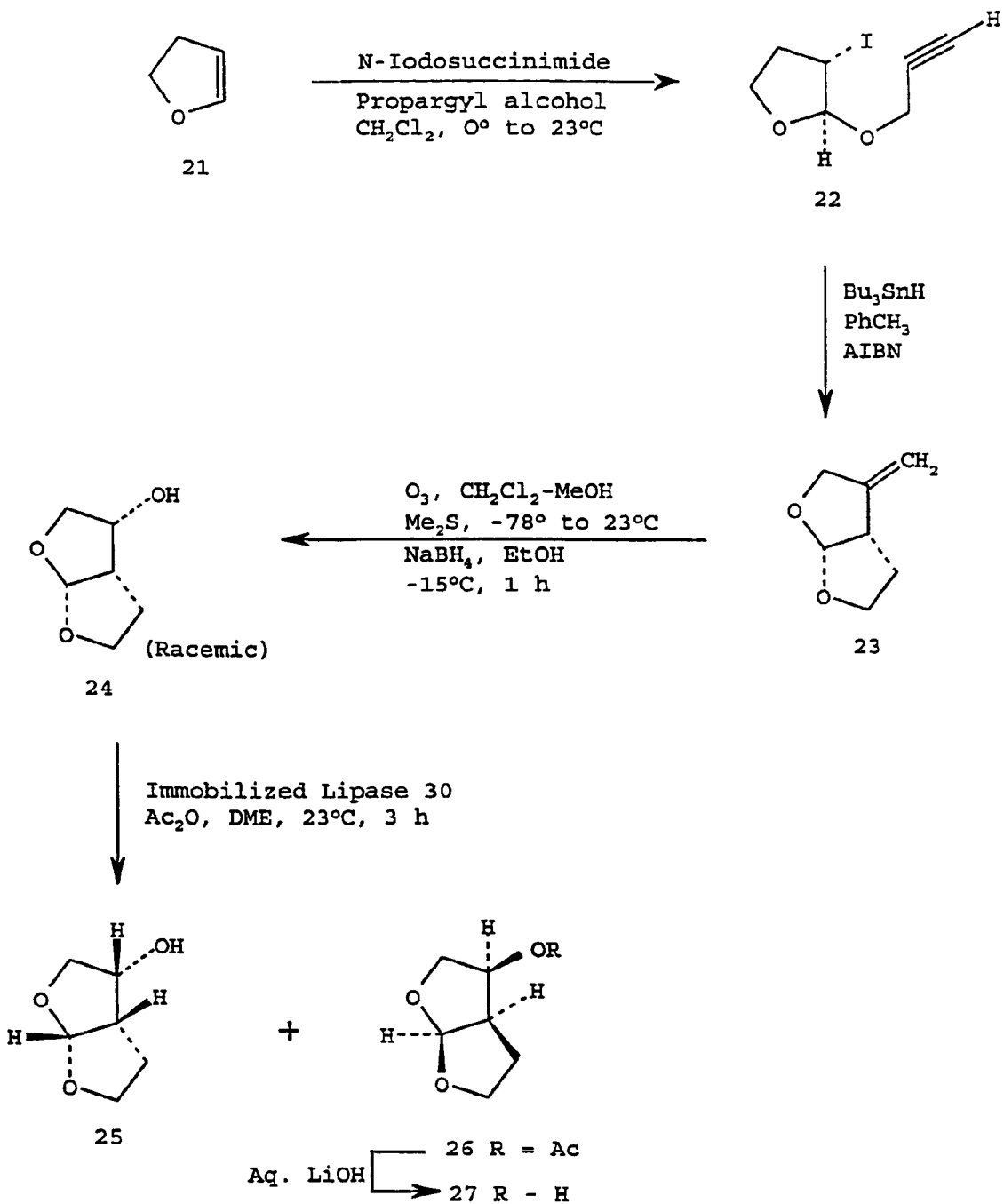


Fig. 2

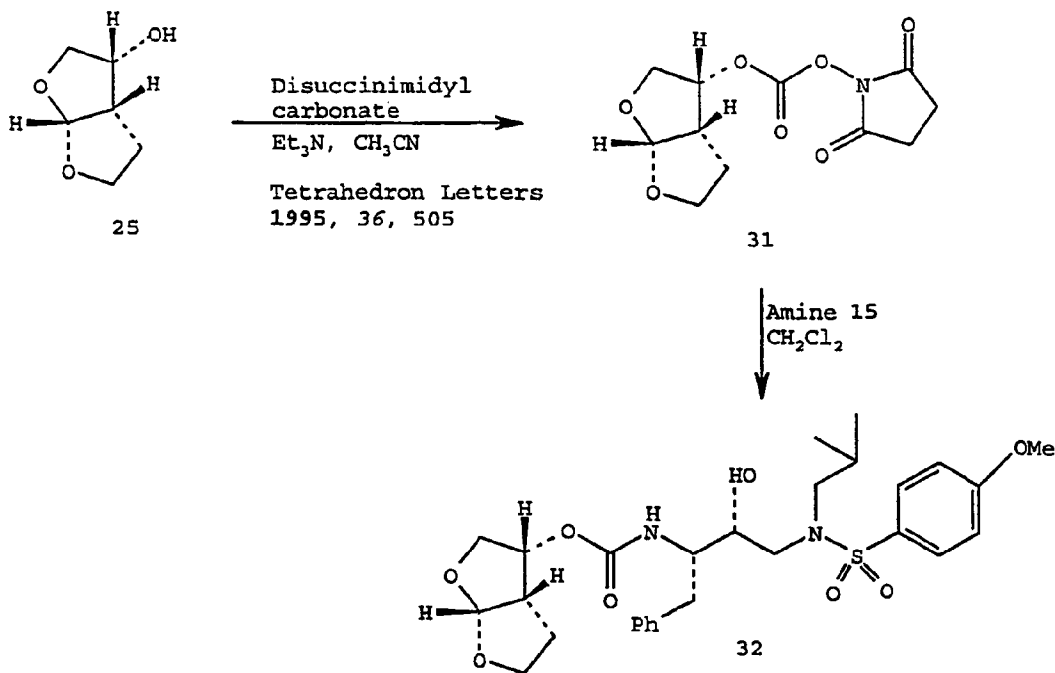


Fig. 3A

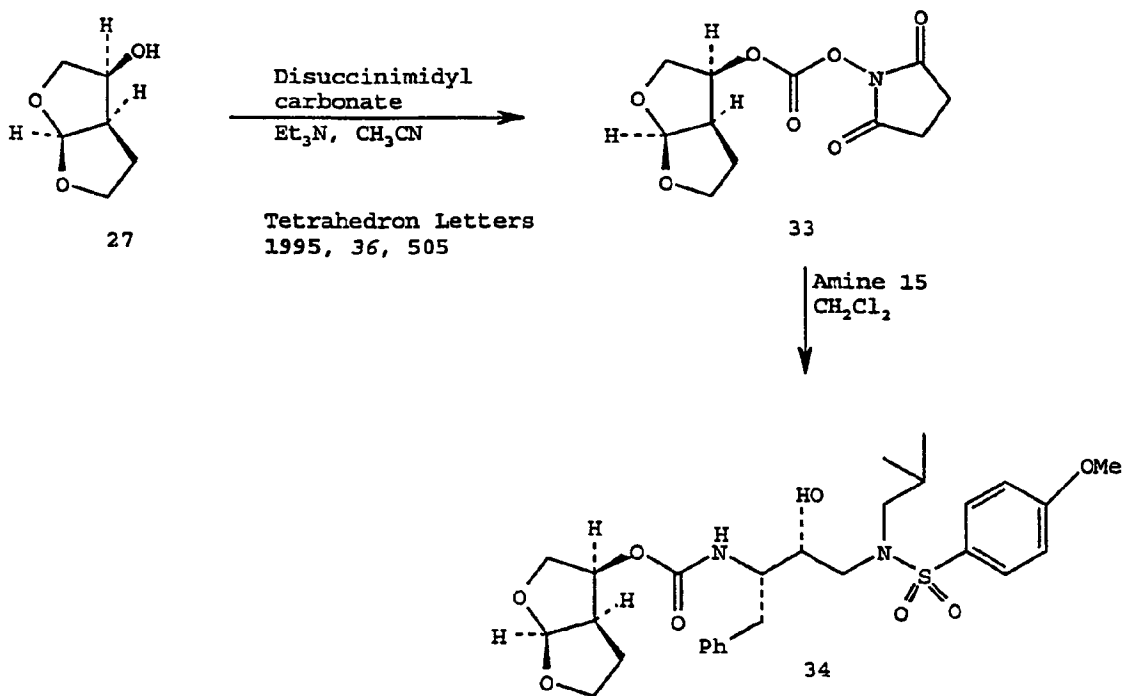


Fig. 3B

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