

Synthesis of C-14 Labeled GABA_A $\alpha 2/\alpha 3$ Selective Partial Agonists and the Investigation of Late-Occurring and Long-Circulating Metabolites of GABA_A Receptor Modulator AZD7325

Markus Artelsmair,¹ Chungang Gu,² Richard Lewis,³ Marc Chapdelaine⁴ and Charles S. Elmore¹

¹ Early Chemical Development, Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden; ² DMPK, Oncology, IMED Biotech Unit, AstraZeneca, Boston, USA; ³ Medicinal Chemistry, Respiratory, Inflammation and Autoimmunity, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden; ⁴ Legacy R&D Wilmington, AstraZeneca, Wilmington DE, USA.

Introduction

GABA (γ -aminobutyric acid) is the main inhibitory neurotransmitter in the mammalian central nervous system. It binds to two main receptor types, namely ionotropic (GABA_A and GABA_C) and metabotropic receptors (GABA_B). GABA_A receptors are pentameric membrane proteins that operate as GABA-gated chloride ion channels. These receptors can be further sub-divided by their subunit architecture, into GABA_A $\alpha 1$ -6, GABA_A $\beta 1$ -3 and GABA_A $\gamma 1$ -3 in humans. GABA_A $\alpha 2$ and GABA_A $\alpha 3$ have been associated with anxiolytic activity, making them of particular interest. Three promising target compounds (see Fig. 1) were identified and required C-14 labeling in order to achieve a better understanding of their DMPK properties.

AZD7325 is a selective GABA_A $\alpha 2$ and $\alpha 3$ receptor modulator intended for the treatment of anxiety through oral administration. A large number of **AZD7325** metabolites were observed across species *in vivo*. This poster presents an interesting metabolic cyclization and aromatization pathway leading to the tricyclic core of **M9** and the oxidative pathways to **M42** and **M10**.

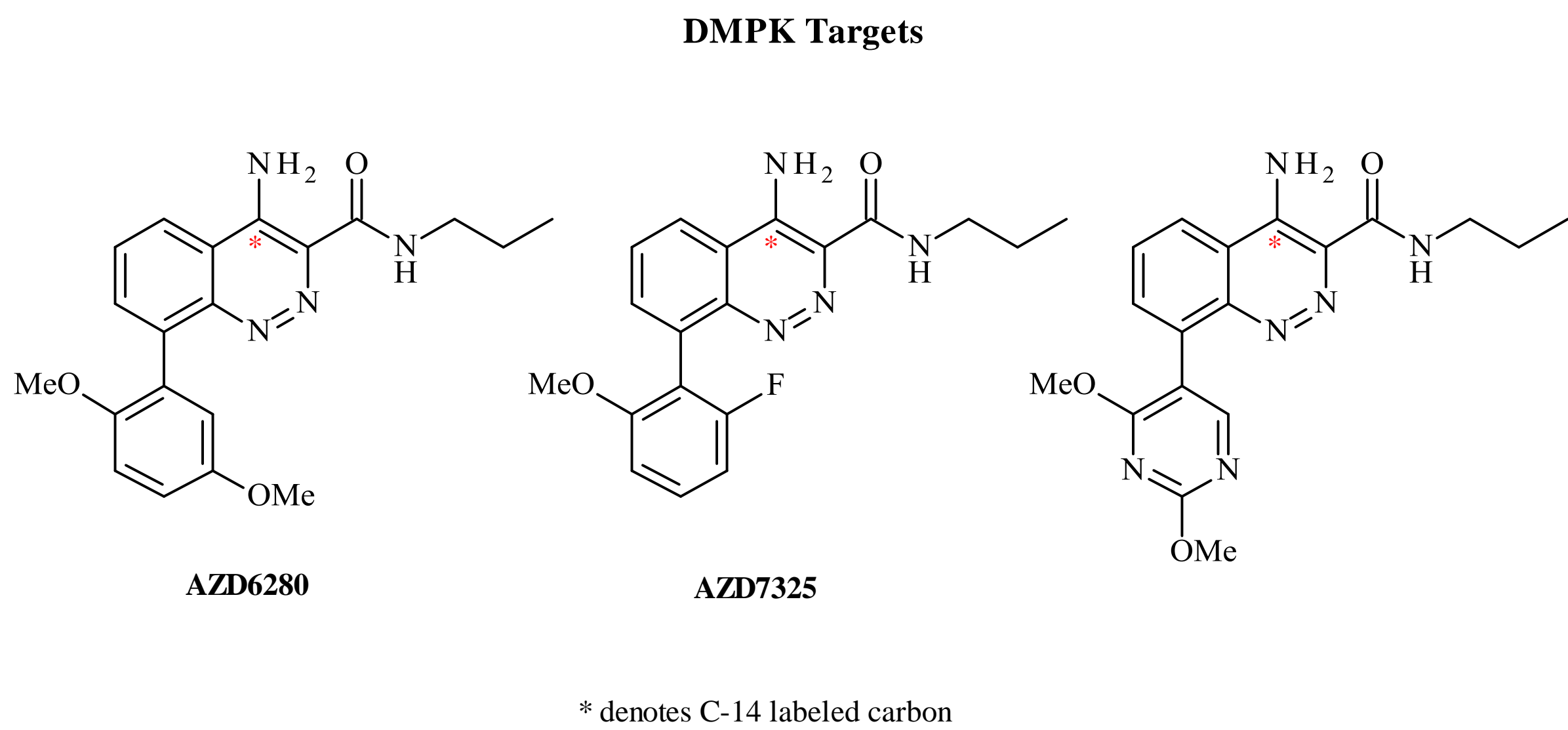


Fig 1. Three drug candidates selected for C-14 labeling to study their DMPK properties.

Synthesis of the key C-14 labeled intermediate

The synthesis of the key intermediate was accomplished by coupling K¹⁴CN with *N*-propyl bromoacetamide to give the corresponding nitrile. This was then converted to an advanced aromatic intermediate in 2 steps *via* the addition of 2-bromodiazobenzene and subsequent cyclisation.

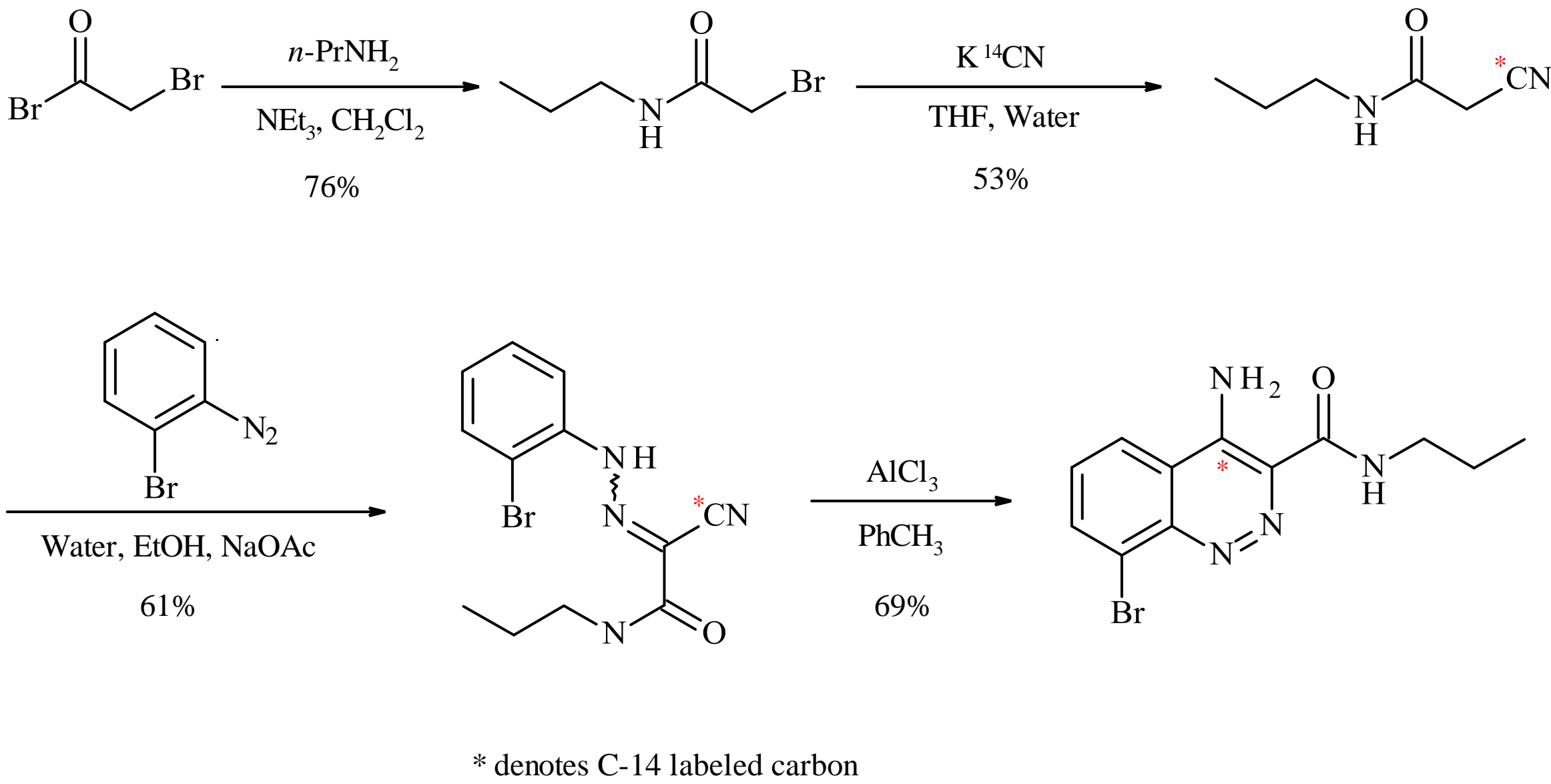


Fig 2. Synthesis of the common precursor for the radiolabeling targets.

Synthesis of C-14 labeled GABA_A $\alpha 2,3$ selective partial agonists

The key intermediate was partitioned to prepare three target compounds *via* Suzuki couplings.

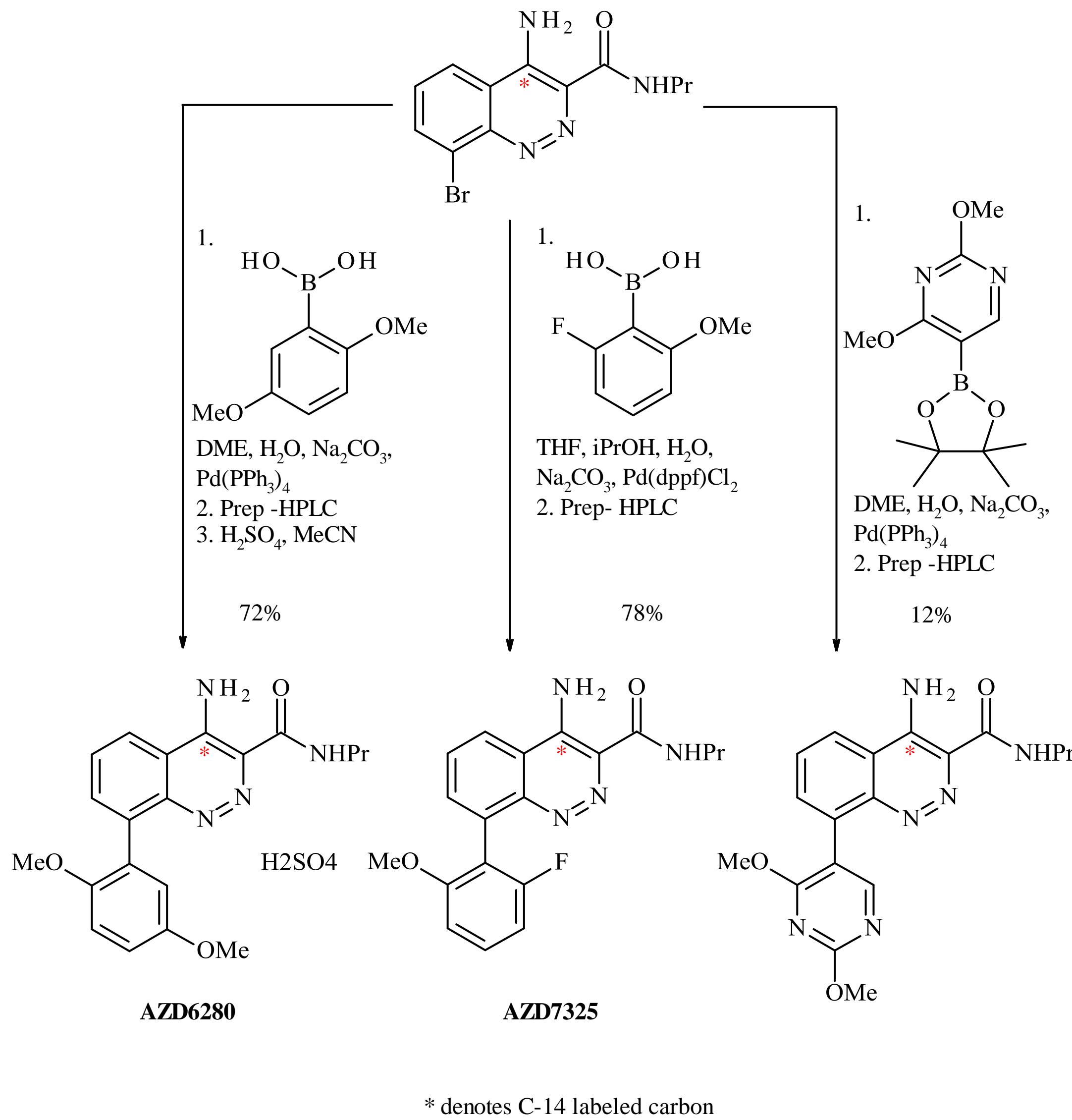


Fig 3. Suzuki coupling reactions used to prepare the radiolabeled drug candidates.

AZD7325 and its metabolites

[¹⁴C]**AZD7325** guided the profiling and identification of a great number of **AZD7325** drug metabolites formed in rat *in vivo*. These were produced through various combinations of oxidations at three sites of the **AZD7325** molecule as well as subsequent conjugation reactions. The insight gained into the rat metabolite profile of [¹⁴C]**AZD7325** and the biotransformation pathways were helpful for the analysis of metabolites of **AZD7325** in human plasma at steady state.

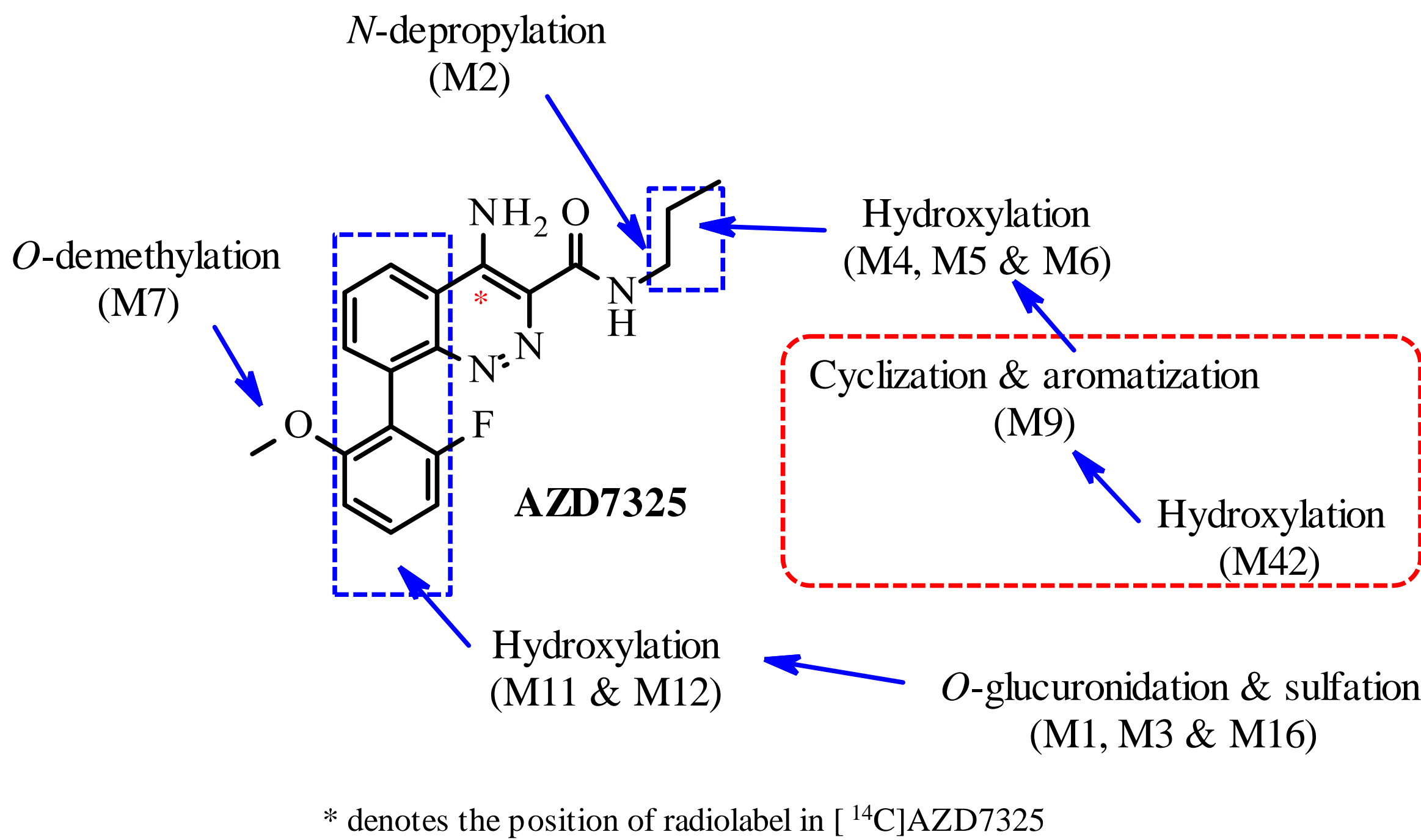


Fig 4. The different sites of metabolism of **AZD7325**.

Metabolic pathways

The metabolism of **AZD7325** was extensive. An interesting metabolic pathway leading to the tricyclic core of **M9** *via* metabolic cyclization and aromatization was observed. Further oxidative metabolism leads to **M42** *via* hydroxylation and **M10** *via* *O*-demethylation. **M9** was only a minor metabolite in human and preclinical animal hepatocytes while **M10** and **M42** were absent from the hepatocytes. These three metabolites were either minor or absent in plasma samples after a single dose, but all became major circulating metabolites after repeated doses in humans and preclinical animals.

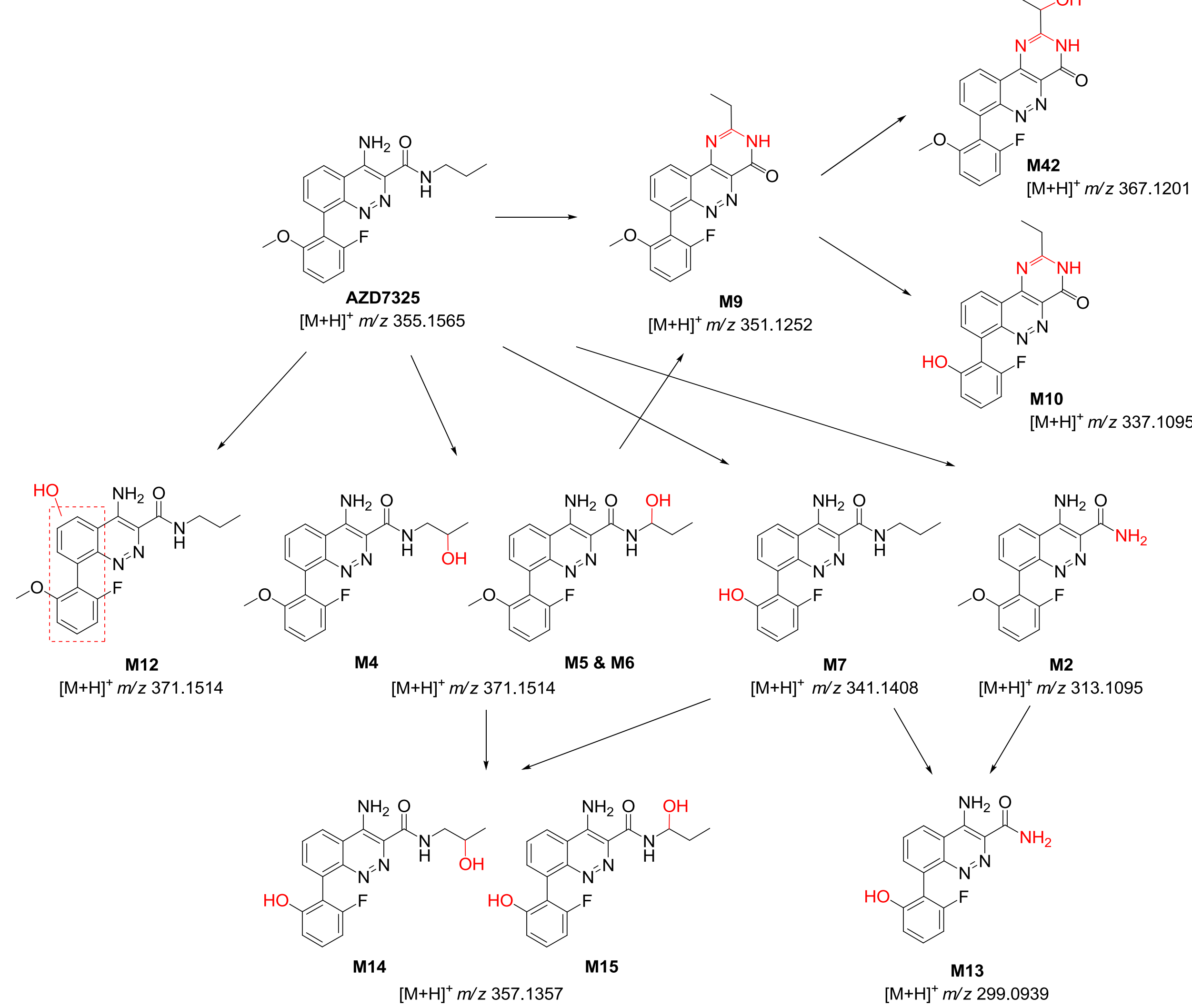


Fig 5. Major metabolites of **AZD7325** elucidated with [¹⁴C]**AZD7325** in rat *in vivo*.

M9 Synthetic standard

An authentic synthetic standard of **M9** was prepared to confirm its novel structure. The standard was then incubated in human liver microsomes, generating **M10** and **M42**. Thereby, **M9** was confirmed as a precursor to **M10** and **M42** *in vitro*.

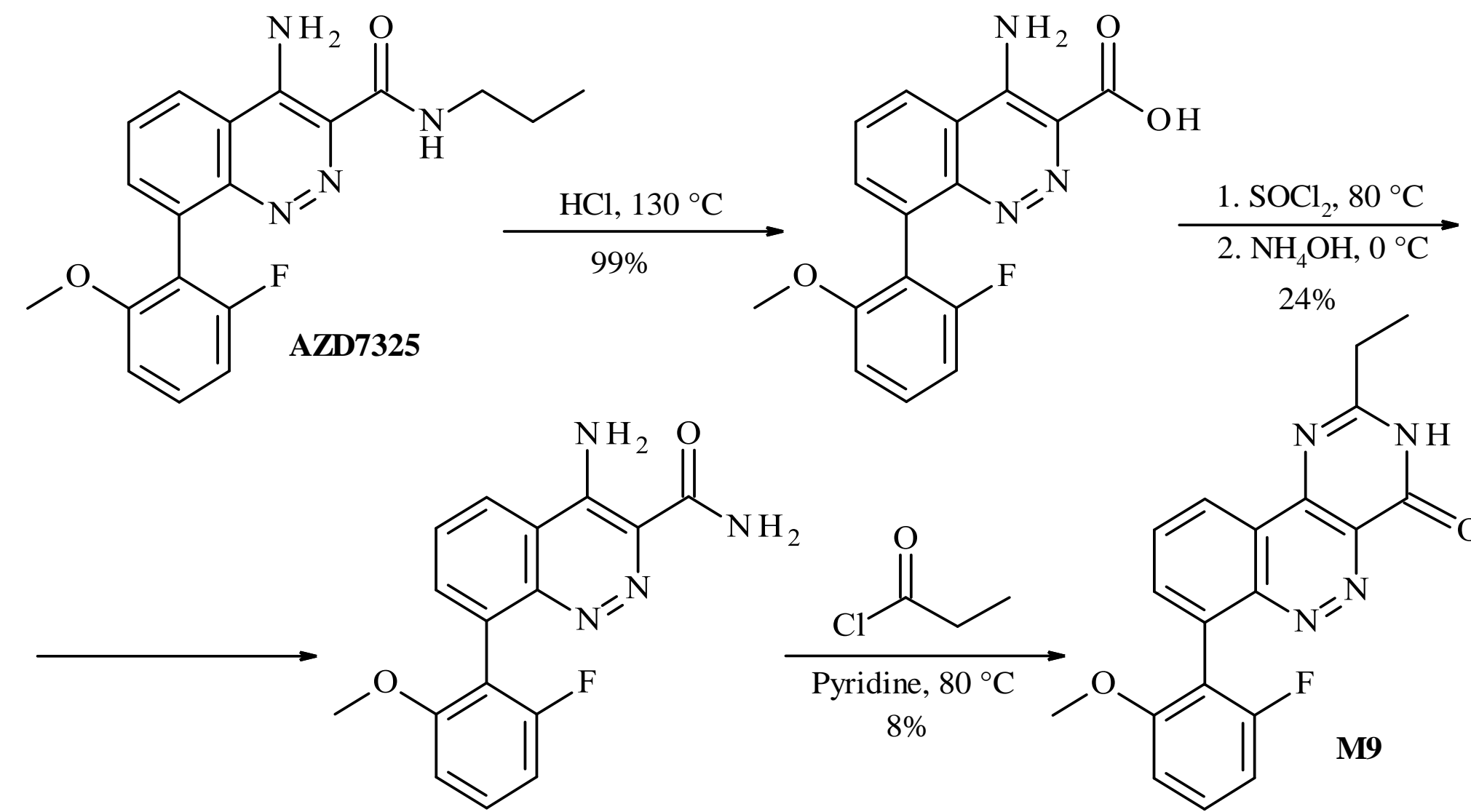


Fig 6. The synthetic route to the authentic standard for **M9**.

Summary

- Several C-14 labeled GABA_A $\alpha 2$ and $\alpha 3$ selective compounds were prepared
- Having an advanced intermediate available allowed for the rapid generation of multiple compounds of this structural class
- Aided by [¹⁴C]**AZD7325** a large number of metabolites were identified for the selected drug candidate
- Chemical synthesis of **M9** confirmed the structure of key metabolites **M9**, **M10** and **M42**



Acknowledgments:

We thank several DMPK colleagues of the AstraZeneca legacy R&D at Wilmington DE, USA for contributions to related *in vitro* studies and bioanalysis. We also thank Dr. James Hall and Dr. Timothy Blake for analytical support.

Three-month safety studies in rat, mouse, and dog were conducted by Charles River Laboratories at Tranent, Edinburgh, in UK.

This work was partially funded from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 675417

