学位論文内容の要旨

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学位論文題名

(Trifluoromethyl) phenyldiazirines in photoaffinity labeling: Improved synthesis, functionalization, and application

（トリフルオロメチルジアジリンによる光アフィニティーラベル：

改良合成、機能化およびその利用）

Photoaffinity labeling (PAL) has emerged as an effective strategy to investigate the interactions between ligands and receptors. Currently, there are three major photophores used in this field: arylazides, benzophenones, and diazirines. Among of them, 3-(trifluoromethyl)-3-phenyldiazirine (TPD) has been used as the most effective photophore due to its thermal and chemical stability, long irradiation wavelength, low rate of rearrangement and high reactivity of its intermediate. In this thesis, my work were carried out around TPD including its improved synthesis, functionalization (deuteration and metabolic study), and further application.

1. Improved synthesis of TPD and corresponding application

Despite of the advantages of TPD and TPD-based photoaffinity labeling probes, their long synthetic procedure are urgent to be solved to further broaden the application of TPD. To shorten the synthesis route of TPD, we developed one-pot syntheses of TPD from corresponding tosyloximes with liquid NH_3 at 80 °C or in the presence of $LiNH_2$ in liquid NH_3 at room temperature. Mechanism investigation indicated that the NH_2^- generated from liquid ammonia or $LiNH_2$ are responsible for the formation of TPD from diaziridine. Numerous TPD derivatives were prepared in good yields including optically pure (trifluoromethyl)diazirinylphenylalanines.

2. TfOD-mediated H/D exchange of cross-linkable aromatic α-amino acids

The combination of deuteration and MS analysis is a useful strategy to analyze α-amino acids and peptides in the field of PAL. Previously, our group reported a hydrogen/deuterium (H/D) exchange for preparing deuterated α-aromatic amino acids and the corresponding peptides with deuterated triflic acid (TfOD). This method has many advantages such as reaction feasibility at low temperature and the controllability

especially the good solubility for α-amino acid derivatives. To further broaden its application, we carried out H/D exchange study for cross-linkable aromatic α-amino acids with TfOD.

3. Metabolic study of photoreactive aromatic α-amino acid with *Klebsiella* sp. CK6

Photoreactive L-phenylalanines are useful building blocks for biological functional analysis of peptides and the corresponding proteins. As a basic work to extend the application scope of photoaffinity labeling to microbiological research, we reported a comparative analysis of the metabolites from inoculation of photoreactive L-phenylalanine derivatives in the presence of *Klebsiella* sp. CK6. The metabolites were identified and compared with that of normal L-phenylalanine and L-tryptophan and the influence factors for the metabolites formation were also discussed in this work.

4. TPD-based photoreactive saccharins for photoaffinity labeling of gustatory receptors

Saccharin is a common artificial sweetener that is hundred times sweeter than sucrose. To prepare photoreactive saccharin is a useful strategy to investigate sweet taste receptors. In this work, we prepared two photoreactive saccharin derivatives containing diazirinyl moiety at the 5- and 6- positions involving the previous one-pot reaction. The syntheses procedures are more convenient and efficient. Further investigation indicated that the photoreactive compounds have enough affinity for the investigation of the sweet and bitter receptors for photoaffinity labeling.

5. Cosolvent-promoted O-benzylation with silver(I) oxide

Silver(I) oxide (Ag_2O)-mediated O-benzylation has emerged as an indispensable strategy in carbohydrates field and synthetic chemistry. While, many reports meet the problems such as the excess use of reagents, preparation of fresh Ag_2O and low reaction yields. To further improve Ag_2O-mediated O-benzylation, we developed an effective cosolvent-promoted O-benzylation strategy with Ag_2O. The cosolvent consisting of CH_2Cl_2 and *n*-hexane can not only improve the reaction solubility for carbohydrates but also increase the benzylation efficiency. The mechanisms were described and its application on the O-benzylation of other carbohydrates and alcohols were performed. Furthermore, TPD was successfully introduced into the 1'-position to prepare a photoreactive sucrose derivative by using the cosolvent-promoted O-benzylation strategy. This component is of great importance to investigate sucrose and its receptor in the field of PAL.