Contribution

A reliable and easy method for synthesis of nitrogen-containing compounds : Ns-strategy and with high-activity trityl type resin

Toshiyuki Kan
Laboratory of Synthetic and Medical Chemistry,
School of Pharmaceutical Sciences, University of Shizuoka,

Tohru Fukuyama
Laboratory of Synthetic Natural Products Chemistry
Graduate School of Pharmaceutical Sciences, University of Tokyo

1. Introduction

Nitrogen-containing compounds are important chemical entities, many of which show unique bioactivity. In view of this their efficient synthesis is an important issue in the development of pharmaceutically active molecule. However, the handling of highly-polar nitrogen-containing compounds is often problematic, and furthermore the propensity of basic nitrogen compounds to undergo oxidation limits the scope of reaction conditions to which these compounds may be submitted. Accordingly, judicious choice of protecting groups for nitrogen-containing groups is an essential part of the planning of any synthesis involving multiple functionalities.[1)] We found that nitrobenzenesulfonyl (Ns) group acts as an active group for the alkylation and succeeded in developing a synthetic procedure involving of the secondary amines by means of this method (Ns-strategy).[2)] Furthermore, the Ns group can be removed under mild conditions. Since we presented this method, it has been widely adopted by many chemists and we have also utilized the process in the total synthesis of bioactive natural products.[3)] In the course of applying the present methodology to the synthesis of the polyamine toxin, we also succeeded in developing a solid phase procedure using trityl resins with much higher loading values than those typically available commercially.[4)] The corresponding solid phase route using this resin represents a significant methodological advance, since no purification steps are required in its execution. In the present articles, we introduce an operationally simple and convenient process for the synthesis of nitrogen-containing compounds based on these methodologies.

2. Synthesis of secondary amines from primary amines

The selective monoalkylation of amines is a non-trivial process and thus no single effective method for they generation of secondary amines from the primary analogue has been established. Scheme 1 shows a number of representative methods for the synthesis of secondary amines and the problems inherent in each approach. For example, the alkylation of primary amine **1** with an alkyl halide or sulfonate ester not only affords the desired secondary amine **2** but frequently also the corresponding, tertiary amine **3** and quarternary ammonium salt **4** arising from over-alkyation. Likewise, the formation of secondary amine **5** by reductive amination of aldehydes or ketones with a suitable reducing agent such as $NaBH_3CN$ can produce tertiary amine **6** as a by-product when the intermediate secondary amine **5** generated in the reaction is sufficiently sterically unencumbered to undergo condensation with a second equivalent of the carbonyl compound and subsequent reduction. Although the synthesis of secondary amines via reduction of the related alkylamide obtained by acylation of the corresponding primary amine with a strong reducing agent such as $LiAlH_4$ and BH_3 is reliable, the harsh conditions required preclude its application to the synthesis of polyfunctional compounds. Recently, the Mitsunobu reaction[5)] involving toluenesulfone (Ts) amide **8a** or trifluoroacetoamide **8b** has been developed. However, since strong basic conditions are required for the deprotection (**9a**,**b**→**2**), its scope of application is limited to those compounds which are stable towards alkaline media.

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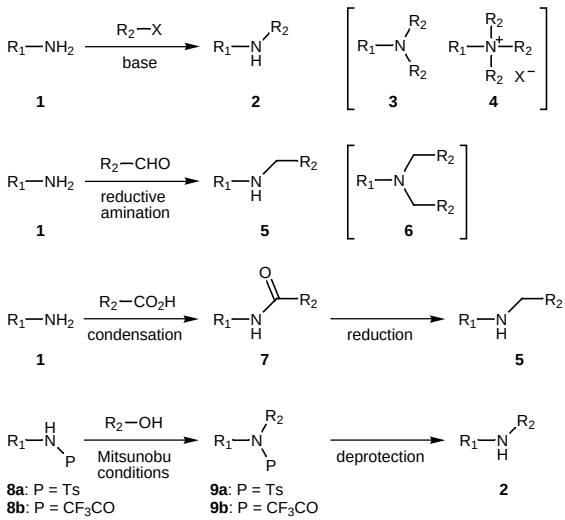
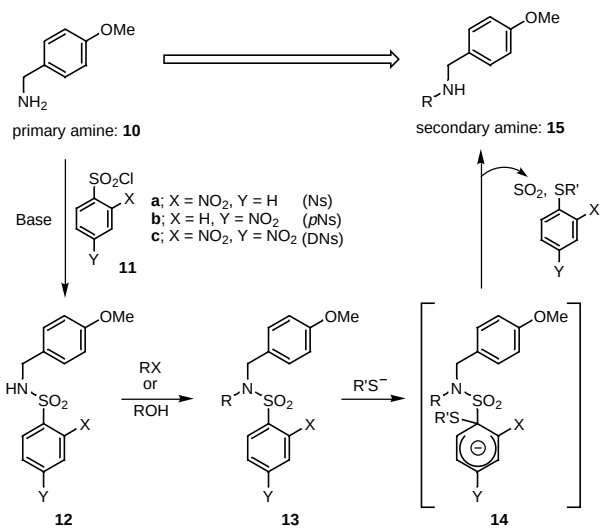
* For convenience, 2-nitro, 4-nitro, and 2,4-dinitro derivatives of nitrobenzenesulfonate will be abbreviated as Ns, *p*Ns and DNs, respectively.



Scheme 1. Conversion of primary amines to corresponding secondary amines.

2.1 2-or 4-Nitrobenzenesulfonamide[2)]

Sulfonamides are a reliable class of nitrogen protecting groups as they are stable under both strong acidic or the more usual basic conditions. Furthermore, sulfonamides carry the advantage that they do not suffer from line broadening in the ^{1}H NMR, which is often seen in the related carbamates and amides. One drawback however is the severe conditions necessary for the removal of toluenesulfonyl (Ts) and methanesulfonyl (Ms) groups which are the most commonly used analogues. We have shown that the related nitrobenzenesulfonamides (Ns) can be converted into the corresponding amine under very mild conditions. In Scheme 2, the reaction of *p*-methoxybenzyl amine (**10**) is shown as a representative example of the "Ns strategy" in which the Ns group is used both as a protecting and activating group for the synthesis of secondary amines from primary amines.

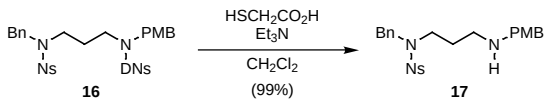


Scheme 2. Conversion of primary amines to corresponding secondary amines via Ns-strategy.

Accordingly, primary amine **10** is allowed to react with 2-nitrobenzenesulfonyl chloride (NsCl) **11a** in the presence of a base affording sulfonamide **12a**. The alkylation of this N-mono-substituted sulfonamide **12a** proceeds readily with an akyl halide (R-X) or the related (R-OH)[6)] under Mitsunobu conditions to produce N-di-substituted sulfonamide **13a**. Nitrobenzenesulfonamide, which has a small pK_a acts as an activating group in the alkylation, and therefore the Mitsunobu reaction which is relatively difficult with Ts amide, proceeds smoothly. When a soft nucleophilic agent (Nu^-) such as PhSH acts on **13a** in the presence of a base, Meizenheimer complex **14a** is formed by the nucleophilic addition of the thiolate anion to an aromatic ring and then it is converted to secondary amine **15** *via* the elimination of SO_2. As mentioned above, the Ns group can be removed under mild conditions and it is also stable under strong acidic and basic conditions. Thus, it is a protecting group for primary or secondary amines, that survives various synthetic reactions. Furthermore, we confirmed that racemization does not take place these series of reaction. We generally use inexpensive NsCl **11a** extensively in our laboratory although 4-nitrobenzenesulfonyl chloride (*p*NsCl:**11b**) offers similar reactivity to that of **11a**.** (Tokyo Chemical Industry Co., Ltd. NsCl: N0142, *p*NsCl: N0144).

2.2 2,4-Dinitrobenzenesulfonamide (DNs)[7)]

2,4-Dinitrobenzenesulfonamide (DNs) group has alkylation ability equal to that of Ns group, but it has a disadvantage that it is unstable when heated for prolonged periods in the presence of a base. However, it is possible to remove only the DNs group selectively since it can be cleaved under milder conditions than those required for the removal of the Ns group. As shown in Scheme 3, the DNs group of diamine **16**, protected by two nitrobenzenesulfonyl groups, could be selectively removed under the conditions of $HSCH_2CO_2H$, Et_3N, affording secondary amine **17** in quantitative yield. In addition, this process has an advantage that 2,4-dinitrophenylthioacetate, formed as by-product, can be removed is removed by separation of the diethyl ether and saturated aqueous sodium bicarbonate solution.

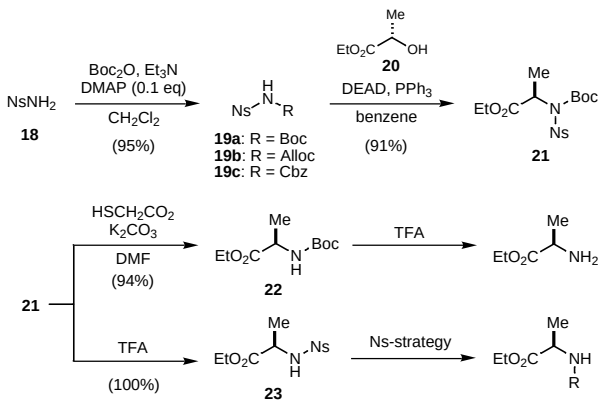


Scheme 3. Selective deprotection of DNs group.

** Although there is no problem about 2-nitrobenzenesulfonamide, complications due to competing side reactions were reported in the deprotection of 4-nitro derivative: P.G.M. Northuis, *Tetrahedron Lett.*, **1998**, *39*, 3889.

3. Synthesis of protected primary amines (N-Carboalkoxynitrobenzenesulfonamide)

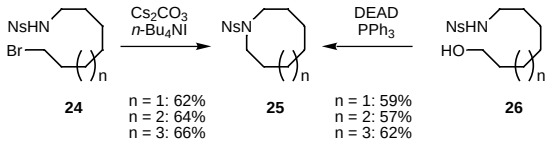
The introduction of a nitrogen atom to alkyl halide or alcohol by nucleophilic substituting reaction to synthesize primary amines is a useful reaction. Although the Gabriel synthesis[9] and azide methods have been developed, there are few general methods for the synthesis of protected primary amines directly. Recently, the Mitsunobu reaction with respect to TsNHBoc by Weinreb *et al.* and the improved Mitsunobu reaction concerning $TsNH_2$ by Tsunoda[10] *et al.* have been reported. We thought that it was possible to develop more useful nitrogen nucleophilic agent by utilizing the characteristics of the Ns group. In $NsNH_2$ which can be easily prepared from ammonium (NH_3) and NsCl **11a**, conversion to the carbamate proceeds in the presence of Et_3N and a catalytic amount of DMAP, giving the Boc derivative of **19a** as a crystalline solid. The sulfonamide **19a** so obtained undergoes smooth alkylation with alkyl halide and Mitsunobu reaction with alcohols. As a representative example, the reaction of **19a** individually with (-)-ethyl lactate **20** is shown in Scheme 4. It is possible to remove the Ns group and Boc group selectively from **21**, which itself was obtained under the usual Mitsunobu reaction conditions. This means that the reaction of thiol with **21** promotes the deprotection of the Ns group, to afford *N*-Boc alanine derivative **22**. Further, the removal of the Boc group from **21** is proceeds under acidic conditions facilitating the conversion to Ns-amide **23**. Primary amines are produced by removing the Boc group of **22**, and **23** can be converted to secondary amine *via* Ns-strategy. In addition, it is possible to synthesize Alloc derivative **19b** and the Cbz derivative **19c** of *N*-Ns-sulfonamide in the same way, Sulfonamide **18** and **19a-c**, which act as crystalline ammonium equivalents, are commercially available from Tokyo Chemical Industry Co., Ltd.



Scheme 4. Alkylation and deprotection of *N*-Boc-Ns-amides.

4. Intramolecular alkylation (synthesis of medium-sized ring compounds)

Recently, many studies on the synthesis of large or medium-sized ring compounds, have been reported using olefin methathesis process. However, there are few studies on the use of direct nitrogen nucleophilic agents in the synthesis of the heterocycles. Accordingly, we applied the alkylation of Ns group to such intramolecular reactions. We found that they displayed usefulness in the cyclization of eight, nine and ten-membered rings - a process that is typically difficult to perform. When Cs_2CO_3 acts on halide **24**, synthesized from sulfonamide **18**, in the presence of *n*-Bu_4NI, the cyclization proceeded smoothly giving heterocycle **25**. In the same way, alcohol **26** was cyclised under Mitsunobu conditions, affording **25** was obtained. It should be noted that cyclization precursors **24** and **26** have no substituents that especially promote the cyclisation and furthermore, the present reaction does not require the high dilution conditions.



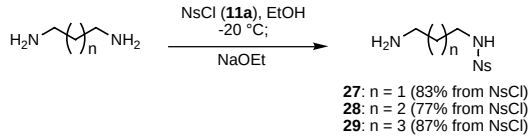
Scheme 5. Construction of medium-sized rings.

5. Synthesis of natural products, polyamine toxin

Recently, a number of natural products having polyamine chain have been isolated with the aid of microanalysis technology. Most of them are often obtained in extremely small amounts from natural source and have strong bioactivity. Accordingly they have attracted a good deal of synthetic interest but there are have been few satisfactory approaches to the construction of the secondary amine portions. We reasoned that the efficient synthesis can be possibly by means of Ns-strategy and launched studies on the synthesis of the natural polyamines.

5.1 Selective protection of diamines

Diamines in which two amino groups are distinguished from each other are not only useful elements for the synthesis of polyamines, but also useful compounds that can be used as linkers of probe molecules *etc*. However, the monocarbamation of symmetric diamines generally proceed in low yield and reactions are notoriously difficult to purify.[13] We have found that Ns protection of diamines on one of the terminal nitrogens selectively. As shown in Scheme 6, monosulfonylation was carried out selectively by adding NsCl slowly to 1,3-diaminopropane at low temperature. The isolation of monosulfonylated diamine **27** can be performed by neutralizing the hydrochloride with NaOEt, filtering off NaCl, evaporation of solvents and removing excess of unreacted diamines under reduced pressure. In addition, it is possible to obtain monosulfonyl species **28** and **29** from both diamines having n=2, 3 in high yield. These diamine derivatives (n=1-5) are also commercially available from Tokyo Chemical Industry Co., Ltd.



Scheme 6. Selective mono-nosylation of diamines.

5.2 Total synthesis of HO-416b[5)]

It is known that certain polyamines isolated from the poison gland of spiders specifically inhibits the excitatory glutamic acid receptor.[14)] This receptor is expected to provide targets lead-compounds for the development of medicines and agrichemicals since it is closely associated with the memory of brain nervous cells and the learning mechanism.[15)] We postulated that the simple synthesis of polyamines could be performed *via* Ns-strategy and launched the studies of synthesizing HO-416b (**30**)[16)] isolated from *Holoena curta*.

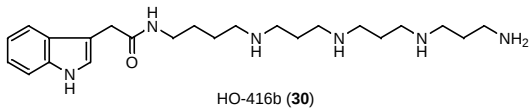
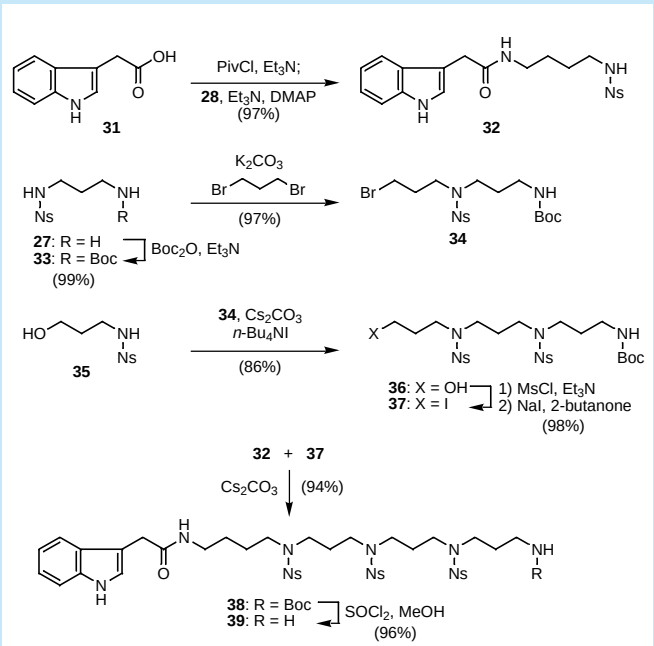


Fig. 1. Structure of HO-416b (**30**).

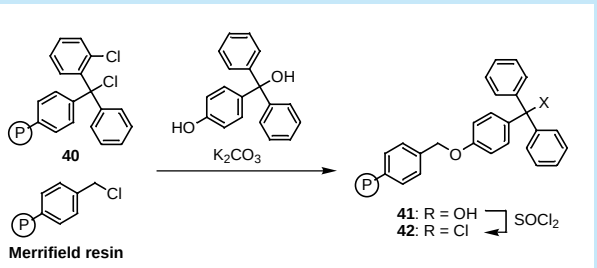
As shown in Scheme 7, a convergent method for the synthesis consisting of the separate construction of the right and left fragments which are subsequently brought together was envisaged. The left fragment **32** was synthesized by the condensation of indoleacetic acid **31** and diamine **28**.



Scheme 7. Total synthesis of HO-416b (**30**).

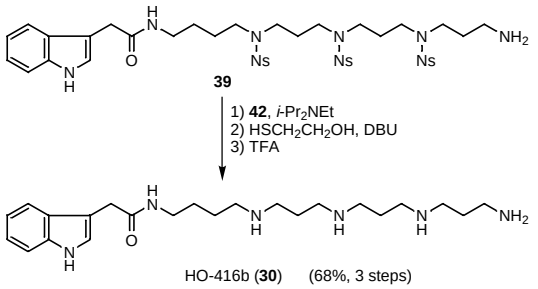
For the right fragment, diamine **33** made by Boc-protection of **27** to Boc was used as starting material. Treatment of this with an excess of 1,3-dibromopropane gave bromide **34**, which was then alkylated with sulfonamide **35** to give **36**. While the coupling with **32** can be also performed by Mitsunobu reaction, we utilized the alkylation of halide due to the ease of purification of the reaction. After mesylation (Ms) of alcohol **36**, the intermediate so obtained was converted to iodine **37** to in preparation for the coupling with **32**. The reaction proceeded smoothly along under basic condition giving couple compound **38** which was deprotected to give primary amine **39**.

Removal of the Ns groups from **39** subsequently gave HO-416b (**30**). However, whilst the removal of the Ns group proceeded without incident, the isolation and purification of **30** was problematic. Generally, reversed-phase HPLC and ion-exchange resin are used for the purification of water-soluble polyamines. However, we thought that excess reagents and nitrobenzene derivatives produced as by-products could be removed by washing, if the deprotection of Ns group was performed on solid phase, so that the purification process was not necessary. Initially we examined the relatively expensive 2-chlorotrityl resin **40** as a support for primary amine **39**, however loading levels were not sufficiently high for efficient reaction. In order to improve loading we designed resin **42** having reaction point far from the polymers (Scheme 8). The reagent may be readily prepared by conversion of trityl alcohol **41** to the corresponding chloride, after alkylation of inexpensive Merrifield resin with *p*-hydroxytrityl alcohol. Resin **42** has high reactivity since trityl cation at the reaction site is stabilized by oxygen atom at the para-position. In addition, this resin can be recovered, regenerated by rechlorination and reused repeatedly repeatedly. Compound **41**, a stable precursor of resin **42**, is also available commercially from Tokyo Chemical Industry Co., Ltd.



Scheme 8. Synthesis of trityl-type resin (**42**).

As shown in Scheme 9, **39** was supported on the solid phase prepared just prior to the utilization by *i*-Pr_2NEt. The deprotection of Ns group on the solid phase was performed under the condition of ($HSCH_2CH_2OH$, DBU). After washing out excess of reagents, the resin was dried and then cut out with (1%, TFA-CH_2Cl_2). Removal of solvent gave the TFA salt of pure HO-416b (**30**). In this way, isolation of high-polar polyamine could be carried without need for purification by performing the last deprotection on solid phase.



Scheme 9. Completion of total synthesis of HO-416b (**30**).

5.3 Total synthesis of 18 membered-ring polyamine, Lipogrammistin-A (43)[17)]

A number of natural products including plant alkaloids having large cyclic polyamine structures are known.[18)] Although many synthetic approaches to these targets have been described the construction of the large ring structural element has proved problematic. Accordingly, we reasoned that the intramolecular alkylation of Ns group, which had proved to be effective for the synthesis of medium membered-ring, could also be useful in the synthesis of large membered-ring amine and launched the synthesis of Lipogrammsitin-A (**43**).

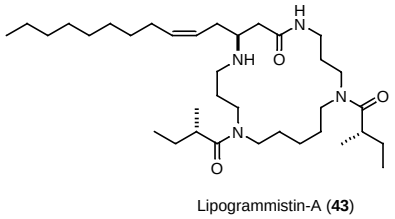
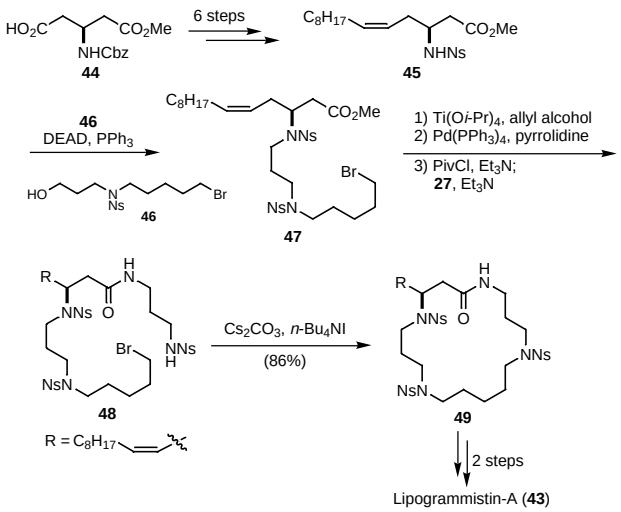


Fig. 2. Structure of Liprogrammistin-A (**43**).

Lipogrammistin-A (**43**) is a polyaminetoxin isolated from the skin of Grammsitidae the structure of which has been determined by a collaborative research of Tachibana, Fusetani *et al*.[19)] This compound has a distinctive structure composed of β-amino acid having 18-membered-ring macrolactam and a long fatty chain. A diagram of our total synthesis involving intramolecular alkylkation of Ns group as the key step is shown in Scheme 10. β-Amino acid derivative **45** was synthesized from the known optically-active carboxylic acid **44** [20)] *via* 6 steps using a Wittig reaction as the central step. Coupling of sulfonamide **45** and alcohol **46** was performed by Mitsunobu reaction to obtain **47**. Since it was found that basic hydrolysis of methyl ester **47** was accompanied by β-elimination of alkylsulfonamide, the corresponding allyl ester was used which could then be deprotected with Pd catalyst without compromising the integrity of the sulfonamide. Carboxylic acid and diamine derivative **27** were condensed according to mixed acid anhydride method to form cyclic precursor **48**. 18-Membered-ring construction, which is a key in the process, was achieved under (Cs_2CO_3, *n*-Bu_4NI) condition. Finally, all of three Ns groups were deprotected and reacted with 2-methylbutanoic acid to achieve the total synthesis of Lipogrammistin-A (**43**).



Scheme 10. Total synthesis of Lipogrammistin-A (**43**).

5.4 Total synthesis of Ephedradine A (50) [21)]

Encouraged by the good results obtained during the total synthesis of Lipogrammistin-A (**43**), our attention turned to the total synthesis study of Ephedradine A (**50**) which is considered to be a more complicated structure. Ephedradine A (**50**) is a spermine alkaloid, isolated as the active ingredient of, Chinese traditional drug *Ephedra sinica Stapfe*. This compound has been known since ancient times although its structure was only determined in 1979 by Hikino at Tohoku University.[22)] The only synthetic approach to the target was conducted by Wasserman in 1985, although their work concentrated on the preparation of the *O*-methyl derivative. The most challenging point of the synthesis of **50** is generally accepted to be the construction of two macrolactam rings in the presence of dihydobenzofuran ring which is unstable to acid and β-amino acid derivative which is unstable to base. On the other hand, since Ns-strategy can be used to promote the cyclization and deprotection under mild conditions, it is possible to construct amine structure using this approach after synthesizing core part **52** as optically-active compound as shown in Fig. 3.

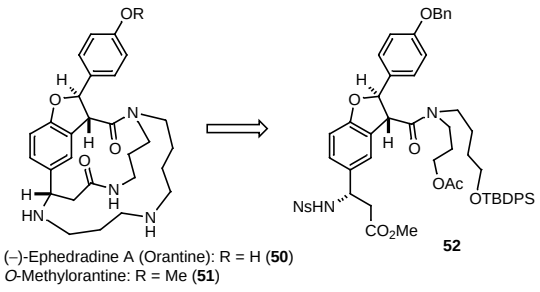
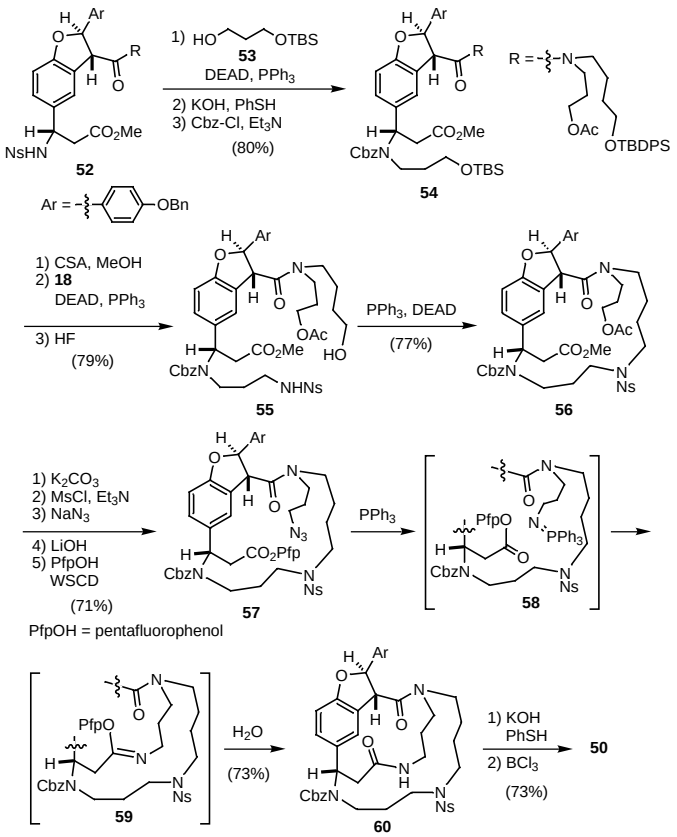


Fig. 3. Structure of (-)-Ephedradine A (**50**).



Scheme 11. Total synthesis of (-)-Ephedradine A (**50**).

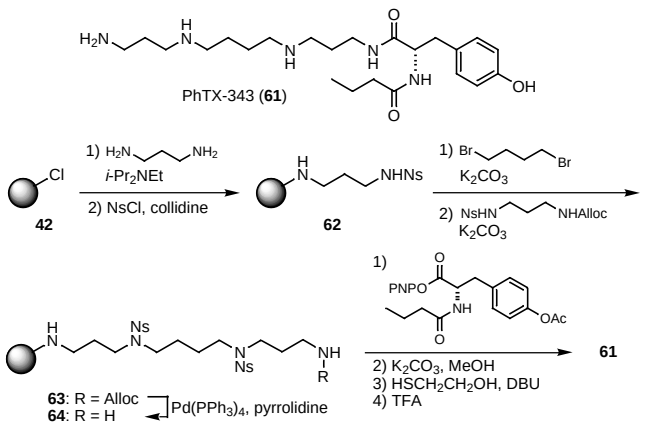
The optically-active dihydrobenzofuran ring of advanced intermediate **52**, shown in Scheme 11, was synthesized by chiral C-H insertion reaction.[24] Subsequently, β-amino ester was constructed by using Sharpless chiral amino hydroxylation reaction[25] as the key step. After introducing the alcohol fragment **53** to sulfonamide **52** *via* the Mitsunobu reaction, protection group on nitrogen atom was converted to Cbz group to obtain **54**. Desilylation followed by condensation of the corresponding alcohol with $NsNH_2$ (**18**), and removal of TBDPS gave the key cyclic precursor **55**. Closure of the 16-membered ring using DEAD and PPh_3 proceeded smoothly to give **56** in good yield. Subsequently, the acetoxy group of **56** was converted to azide and then the methyl ester was converted to pentafluorophenyl (PfpOH) ester **57**. Subsequent Staudinger reaction[26] of **57** with PPh_3 proceeded without incident to produce imino-phospholane **58** which was united brought together by intramolecular aza-Wittig reaction with the active ester affording imino ether **59**. Hydrolysis of this compound was performed to give to macrolactam **60**. Finally, simultaneous removal of the Ns groups of **60** along with the benzyl group and Cbz group using BCl_3 accomplished the total synthesis of Ephedradine A (**50**). The present total synthesis revealed power and functional group tolerance of the Ns-strategy and allowed us to demonstrate new chemistry for the creation of amido bond.[27]

6. Solid phase synthesis by highly active trityl type resin (42)

The trityl type resin **42** developed by us has not only a high loading efficiency of amines, but also permits alkylation of Ns amide and alkyl halide, and amidation reaction as well as the reaction with secondary amine on solid phase. This discovery opened the door to new combinatorial procedures described below.

6.1 Solid phase synthesis of PhTX-343 (61)

Polyamines such as spermine and spermidine are physiologically active substances ubiquitous in living systems and a number of compounds containing them are also known.[29] Therefore, an easy method for synthesizing various compounds are strongly desired. This process can be facilitated by using a reaction sequence utilizing resin **42** which does not require purification in the final step. If alkylation on solid phase is realized, it would lead to a successful combinatorial synthesis of the desired compounds could be realized. In order to demonstrated the efficacy of our methodology, we launched the synthesis of a spermine derivative, philanthotoxin-343 (PhTX-343: **61**).[30] The outline of the synthesis is shown in Scheme 12. After linking diaminopropane to resin **42**,



Scheme 12. Solid-phase synthesis of PhTX-343 (**61**).

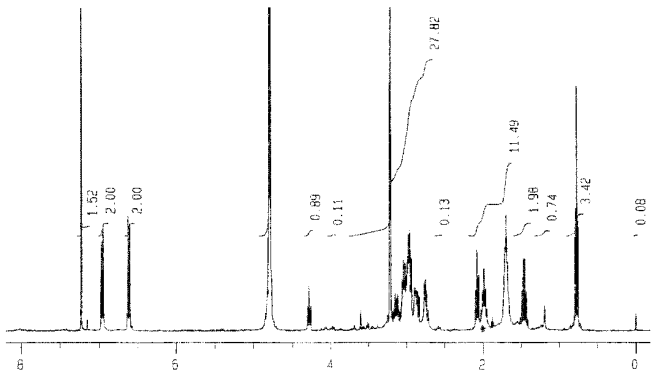
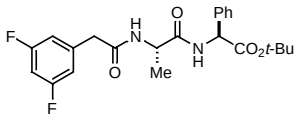


Fig. 4. ^{1}H NMR spectra of synthetic PhTX-343 (**61**).

the free amine was protected with Ns group to prepare Ns-amide **62**. Subsequently protected spermine derivative **62** could be readily synthesised by alkylation of sulfonamide with dibromobutane in an iterative sequence. According to this process, it is possible to combine various diamines and halides and it is also easy to perform Mitsunobu reaction with alcohols. Thus, it is easy to synthesize a library of polyamine chains having different length. In addition, facile removal from **64** gave the corresponding terminal amine which could be readily alkylated or acylated. Hence, it is a useful intermediate for the synthesis of spermine-linked compounds. In the synthesis of PhTX-343 (**61**), tyrosine derivative was introduced to **64** and Ns group was removed and the compound cleaved from the solid phase to achieve the total synthesis *via* nine steps from the resin **42** and in a total yield of 75%. Fig. 4 shows ^{1}H NMR of the material obtained simply by removal of solvent after the final step. In the present synthesis, it was possible to obtain a pure product as shown in Fig. 4 using a sequence which did not require purification at any stage. In this way, we succeeded in developing the revolutionary synthesizing process by combining our solid phase **42** with Ns-strategy.

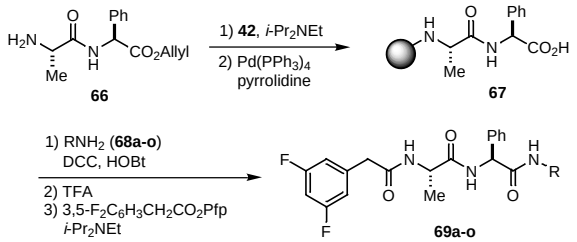
6.2 Parallel synthesis of dipeptide type γ-secretase inhibitor DAPT derivative[31)]

In our laboratory, we have conducted research on the development of a γ-secretase inhibitor[32)] important to the retardation of the onset of Alzheimer's disease and the clarification of its function, as a collaborative research project of the laboratory for several years.[33)] As a part of the research, we have performed structure-activity studies on DATP (**65**) [34)] reported by Elan Co., Ltd. Group as an inhibitor by the group of Elan Co., Ltd., and discovered almost equal activity of the compound, of which C-terminal *t*-Bu ester is converted to amide to that of **65**.



N-[*N*-(3,5-difluorophenylacetyl)-L-alanyl]-(*S*)-phenylglycine-*tert*-butyl ester (DAPT **65**)

Fig. 5. Structure of DAPT (**65**).



R = n-C_7H_{15} (**a**), CH_2Ph (**b**), $CHPh_2$ (**c**), $CH_2C_6H_4$-*o*-OMe (**d**), $CH_2C_6H_4$-*m*-OMe (**e**), $CH_2C_6H_4$-*p*-OMe (**f**), $CH_2C_6H_4$-*o*-Cl (**g**), $CH_2C_6H_4$-*m*-Cl (**h**), $CH_2C_6H_4$-*p*-Cl (**i**), $CH_2C_6H_4$-*o*-Br (**j**), $CH_2C_6H_4$-*m*-Br (**k**), $CH_2C_6H_4$-*p*-Br (**l**), $CH_2C_6H_4$-*p*-F (**m**), $CH_2C_6H_4$-*p*-CO_2Me (**n**), $CH_2C_6H_4$-*p*-Bz (**o**).

Scheme 13. Parallel synthesis of DAPT derivatives (**69a-o**).

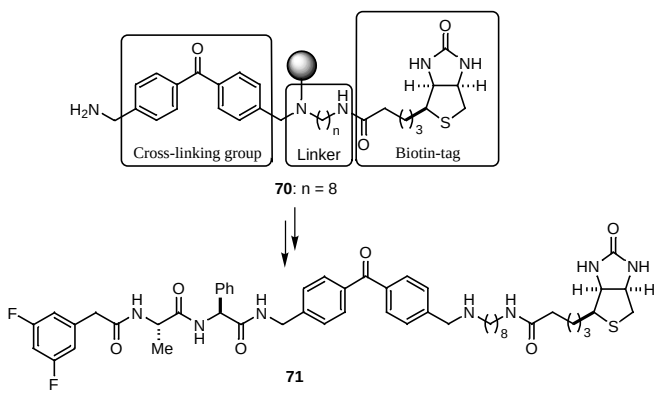
Accordingly, we attempted the parallel synthesis on solid phase with resin **42** in order to synthesize a range of DAPT (**65**) amide compounds bearing various C-terminal substituents. After supporting dipeptide **66** on solid phase resin, the allyl ester was removed to afford carboxylic acid **67**. The condensation of carboxylic acid **67** with various kinds of amines **68 a-o** progressed smoothly using DOC in the presence of an excess of HOBt. Subsequent cleavage from the solid phase resin was carried out under acidic condition, and the phenyl acetic acid segment was subsequently introduced according to the active ester method. Accordingly, we succeeded in obtaining derivatives **69 a-o** having amide, which were converted from C-terminal of DAPT (**65**) only by filtering and washing crystals of crude products after the reaction.

In general, the solid phase synthesis of peptide represented by the Merrifield method is performed by linking the C-terminal to the solid phase, and repeating the condensation and deprotection with N-protected amino acid. On the contrary, according to the present synthetic process, the condensation proceeds without epimerization at the α-position in using *N*-acylamino acids whose N-terminal is linked. Thus, it is possible to extend the peptide chain from N-terminal to C-terminal. Further, due to the fact that amido DAPT derivative **69o** had the activity 30 times stronger activity than DAPT (**65**) itself, this technique was efficacious in the development of the probe.

6.3 Solid phase synthesis of photoaffinity probe[35)]

In recent studies on familial Alzeheimer's disease, presenilin (PS) was cloned as one of genes causing the disease. Later, it was revealed that γ-secretase itself is a huge complex mainly composed of membrane protein produced by the Ps gene.[36)] The photoaffinity labeling method is one of a few effective analytical methods, although the biochemical analysis of membrane protease is difficult.[37)] For the probe synthesis, it is necessary to introduce a photoreactive group which is cross-linked with compounds having a strong affinity with protein (high activity) by exposure to light. Benzophenone is a frequently used photoreactive group for the photoaffinity labeling. Thus, it was preferable to increase the activity of the derivative **69o** showing higher activity than DAPT (**65**). Further, the adoption of the biotin tag makes it possible to realize the easy detection and purification of protein by using the high biotin affinity with avidin. Accordingly, we synthesized photoreactive benzophenon and biotin, and simultaneously synthesized solid phase resin **70** into which they could be readily introduced.

We used mono Ns diamine synthesized according to the process above mentioned as linker. After it was linked to benzophenone by Mitsunobu reaction and condensed with biotin and then secondary amine was by deprotection of Ns group. The secondary amines so obtained were



Scheme 14. Solid-phase synthesis of photoaffinity probe (**71**).

supported on solid phase resin **42** so as to succeed in synthesizing benzophenone-biotin unit type solid phase resin **70** having an amino group reactive with ligand at the terminal. Subsequently, after condensation of carboxylic acid derivative of DAPT(**65**) and amine **70**, we achieved the synthesis of photoaffinity probe **71** by cleavage from the solid phase under acidic conditions. In this case, complicated purification process was not necessary similarly with the cases of the synthesis of **30** and **61**. In addition, it is often difficult to purify the compounds to which biotin is introduced because they become insoluble in organic solvent. We believed that this synthetic strategy is also of great significance from this point of view. In fact, we have succeeded in detection of **23** and **26** kDa proteins derived from presenilin, that disappear in the presence of DAPT (**65**) in the photoaffinity-labeling experiment with the probe **71**.[38,39)]

7. Conclusion

For many pharmacologically active compounds such as pharmaceutical products medicines functioning within living body, solubility becomes an important and serious problem because the environment in which they function is aqueous. In general, compounds having nitrogen atom exist in many medicines because they show high solubility in water. However, as outlined in the current report it is generally acknowledged by synthetic chemists that the generation of compounds containing nitrogen atom is often problematic. We believe that use of the Ns group offers a potential solution to these problems and have developed a wide range of protocols which utilize it. In the process, we have shown that the Ns group worked both on the protection (defence) and on the activation of the alkylation (attack). Thus, the total synthesis of some natural products and the contribution to the development of drugs such as those of Alzeheimer's disease could be realized. In conclusion, we hope and expect that the chemistry introduced in the present contributed article is will be widely used in the chemical community.

The studies referenced in the present article were conducted at the Laboratory of Natural Products Synthetic Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo. We express our sincere thanks to self-sacrificing efforts of all the students, described in the references. In addition, the research on γ-serectase, introduced in the last section of the report was carried out in collaboration with the Laboratory of Neurophotology and Neuroscience Laboratory of Rational Medical Science, Graduate School of Pharmaceutical Sciences, University of Tokyo. We would also like to thank Professor Takeshi Iwatsubo and Professor Hideaki Natsukari for giving to us a chance of taking on studies in fields other than total synthesis.

Reference

1) T. W. Green, P. G. Wuts, "Protective Groups in Organic Synthesis" 3rd ed., John Wiley and Sons, Inc., New York, 1998, p. 494.
2) (a) T. Fukuyama, C.-K. Jow, M. Cheung, *Tetrahedron Lett.*, **1995**, *36*, 6373. (b) W. Kurosawa, T. Kan, T. Fukuyama, *Org. Synth.*, **2002**, *79*, 186.
3) Review articles on Ns-strategy, T. Kan, T. Fukuyama, *Yuki Gosei Kagaku Kyokaishi (J. Synth. Org Chem. Jpn.)*, **2001**, *59*, 779; (b) T. Kan, T. Fukuyama, *Chem. Commun.*, **2004**, 353.
4) (a) Y. Hidai, T. Kan, T. Fukuyama, *Tetrahedron Lett.*, **1999**, *40*, 4711. (b) Y. Hidai, T. Kan, T. Fukuyama, *Chem. Pharm. Bull.*, **2000**, *48*, 1570.
5) (a) J. R. Henry, L. R. Marcint, M. C. McIntosh, P. M. Scola, G. D. Harris, S. M. Weinreb, *Tetrahedron Lett.*, **1989**, *30*, 5709. (b) T. Tsunoda, J. Otsuka, Y. Yamashita, S. Ito, *Chem. Lett.*, **1994**, 539.
6) Review articles on Mitsunobu reaction: (a) O. Mitsunobu, *Synthesis*, **1981**, 1. (b) D. L. Hughes, *Org. React.*, **1992**, *42*, 335.
7) T. Fukuyama, M. Cheung, C.-K. Jow, Y. Hidai, T. Kan, *Tetrahedron Lett.*, **1997**, *38*, 5831.
8) T. Fukuyama, M. Cheung, T. Kan, *Synlett*, **1999**, 1301.
9) Review articles on Gabriel method: M. S. Gibson, R. W. Bradshaw, *Angew. Chem., Int. Ed.*, **1968**, *7*, 919.
10) (a) T. Tsunoda, H. Yamamoto, K. Goda, S. Ito, *Tetrahedron Lett.*, **1996**, *37*, 2457. (b) Current review articles on the method of Tsunoda *et al.*: *TCIMAIL*, **2004**, number *123*, 2.
11) (a) T. Kan, H. Kobayashi, T. Fukuyama, *Synlett*, **2002**, 697. (b) T. Kan, A. Fujiwara, H. Kobayashi, T. Fukuyama, *Tetrahedron*, **2002**, *58*, 6267.
12) Review articles on natural polyamine: A. Guggisberg, M. Hesse, "The Alkaloids", Vol. 50, eds. by Cordell G. A., Brossi H. S., Academic Press, New York, 1998, p. 219.
13) W. J. Fiedler, M. Hesse, *Helv. Chim. Acta*, 76, 1511 (1993).
14) Review articles on spider venom (isolation, decision of structure, synthesis): A. Schafer, H. Benz, W. Fiedler, A. Guggisberg, S. Bienz, M. Hesse, "The Alkaloids", Vol. 45, ed. by Cordell G. A., Academic Press, New York, 1994, p. 1.
15) Review articles on spider venom (pharmacology): A. L. Mueller, R. Roeloffs, H. Jackson, "The Alkaloids", Vol. 46, eds. by Cordell G. A., Brossi H. S., Academic Press, New York, 1994, p. 63.
16) G. B. Quistad, C. C. Reuter, W. S. Skinner, P. A. Dennis, S. Suwanrumpha, E. W. Fu, *Toxicon.*, **1991**, *29*, 329.
17) A. Fujiwara, T. Kan, T. Fukuyama, *Synlett*, **2000**, 1667.

18) Review articles on cyclic polyamines: A. Guggiserberg, M. Hesse, “The Alkaloids”, Vol. 22, 1983, p. 85.
19) (a) H. Onuki, K. Tachibana, N. Fusetani, *Tetrahedron Lett.*, **1993**, *34*, 5609. (b) H. Onuki, K. Ito, Y. Kobayashi, N. Matsumori, K. Tachibana, N. Fusetani, *J. Org. Chem.*, **1998**, *63*, 3925.
20) M. Ohno, S. Kobayashi, T. Iimori, Y.-F. Wang, T. Izawa, *J. Am. Chem. Soc.*, **1981**, *103*, 2405.
21) W. Kurosawa, T. Kan, T. Fukuyama, *J. Am. Chem. Soc.*, **2003**, *125*, 8112.
22) (a) M. Tamada, K. Endo, H. Hikino, C. Kabuto, *Tetrahedron Lett.*, **1979**, *20*, 873. (b) P. Dätwyler, H. Bosshardt, S. Johne, M. Hesse, *Helv. Chim. Acta*, **1979**, *62*, 2712.
23) H. H. Wasserman, R. K. Brunner, J. D. Buynak, C. G. Carter, T. Oku, R. P. Robinson, *J. Am. Chem. Soc.*, **1985**, *107*, 519.
24) W. Kurosawa, T. Kan, T. Fukuyama, *Synlett*, **2003**, 1028.
25) G. Li, H. H. Angert, K. B. Sharpless, *Angew. Chem. Int. Ed.*, **1996**, *35*, 2813.
26) H. Staudinger, J. Meyer, *Helv. Chim. Acta*, **1919**, *2*, 635.
27) H. Fuwa, Y. Okamura, Y. Morohashi, T. Tomita, T. Iwatsubo, T. Kan, T. Fukuyama, H. Natsugari, *Tetrahedron Lett.*, **2004**, *45*, 2323.
28) T. Kan, H. Kobayashi, T. Fukuyama, *Synlett*, **2002**, 1338.
29) Review articles on polyamines and their coupled body: (a) G. Karigiannis, D. Papaioannou, *Eur. J. Org. Chem.*, **2000**, *65*, 1841. (b) V. Kuksa, R. Buchan, *Synthesis*, **2000**, 1189.
30) A. T. Eldefrawi, M. E. Eldefrawi, K. Konno, N. A. Mansour, K. Nakanishi, E. Oltz, P. N. Usherwood, *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 4910.
31) T. Kan, Y. Tominari, K. Rikimaru, Y. Morohashi, H. Natsugari, T. Tomita, T. Iwatsubo and T. Fukuyama, *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 1983.
32) Recent review articles on secretase: M. S. Wolfe, *Nature. Rev. Drug. Discov.*, **2002**, *1*, 859.
33) Y. Takahashi, I. Hayashi, Y. Tominari, K. Rikimaru, Y. Morohashi, T. Kan, H. Natsugari, T. Fukuyama, T. Tomita, T. Iwatsubo, *J. Bio. Chem.*, **2003**, *278*, 18664.
34) H. F. Dovey, V. John, J. P. Anderson, L. Z. Chen, P. de Saint Andrieu, L. Y. Fang, S. B. Freedman, B. Folmer, E. Goldbach, E. J. Holsztynska, K. L. Hu, K. L. Johnson-Wood, S. L. Kennedy, D. Kholodenko, J. E. Knops, L. H. Latimer, M. Lee, Z. Liao, I. M. Lieberburg, R. N. Motter, L. C. Mutter, J. Nietz, K. P. Quinn, K. L. Sacchi, P. A. Seubert, G. M. Shopp, E. D. Thorsett, J. S. Tung, J. Wu, S. Yang, C. T. Yin, D. B. Schenk, P. C. May, L. D. Altstiel, M. H. Bender, L. N. Boggs, T. C. Britton, J. S. Clemens, D. L. Czilli, D. K. Dieckman-MacGinty, J. J. Droste, K. S. Fuson, B. D. Gitter, P. A. Hyslop, E. M. Johnstone, W. Y. Li, S. P. Little, T. E. Mabry, F. D. Miller, J. E. Audia, *J. Neurochem*, **2001**, *76*, 173.
35) T. Kan, Y. Tominari, Y. Morohashi, H. Natsugari, T. Tomita, T. Iwatsubo, T. Fukuyama, *Chem. Commun.*, **2003**, 2244.
36) N. Takasugi, T. Tomita, I. Hayashi, M. Niimura, Y. Takahashi, G. Thinakaram, T. Iwastubo, *Nature*, **2003**, *422*, 438.
37) Review articles on photoaffinity labeling: (a) F. Kotozyba-Hilbert, I. Kapfer, M. Goeldner, *Angew. Chem., Int. Ed. Engl.*, **1995**, *34*, 1296. (b) G. Dorman, G. D. Prestwich, *Biochemistry*, **1994**, *33*, 5661.
38) Y. Morohashi, T. Kan, Y. Tominari, H. Fuwa, Y. Okamura, N. Watanabe, H. Natsugari, T. Fukuyama, T. Iwatsubo, T. Tomita, *J. Biol. Chem.*, **2006**, *281*, 14670.
39) H. Fuwa, Y. Takahashi, Y. Konno, N. Watanabe, H. Miyashita, M. Sasaki, H. Natsugari, T. Kan, T. Fukuyama, T. Tomita, T. Iwatsubo, *ACS Chemical Biology*, **2007**, *2*, 408.

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Introduction of authors

Toshiyuki Kan

Professor, Laboratory of Synthetic Organic and Medicinal Chemistry, School of Pharmaceutical Science, University of Shizuoka

[Brief career history] 1986, Graduated from Undergraduate School, Faculty of Science, Department of Chemistry, Hokkaido University. 1993, Completed doctoral course of Research Faculty of Science (supervisor, Professor Haruhisa Shirahama). 1993-1996, Scientist of Suntory Institute for Bioorganic Research. 1996, Assistant Professor of Faculty of Pharmaceutical Science, the University of Tokyo. 2004, Associate Professor of Graduate School of Pharmaceutical Science, since 2005, Present post, 2002, Incentive award from the Society of Synthetic Organic Chemistry, Japan. 2004, Pharmaceutical Research Vision Award from the Pharmaceutical Society of Japan.
[Specialty] Synthetic organic chemistry, Chemical Biology

Tohru Fukuyama

Professor, Graduate School of Pharmaceutical Science, University of Tokyo

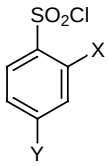
[Brief career history] 1971, Graduated from school of Agricultural, Department of Agricultural Chemistry, Nagoya University. 1977, Ph.D from Department of Chemistry, Harvard University. 1977-1978, Postdoctoral Fellow of Graduate School of Harvard University. 1978, Assistant Professor, Department of Chemistry, Rice University. 1982, Associate Professor of Rice University. 1988, Professor of Rice University. 1995, Professor, Faculty of Pharmaceutical Sciences, University of Tokyo. Since 1997, Present post. 1993, Arthur C.Cope Scholar Award from American Chemical Society. 2002, Academic Award from the Society of Synthetic Organic Chemistry, Japan. 2003, Senior Award from International Society of Heterocyclic Chemistry. 2004, Arthur C. Cope Award from American Chemical Society. 2006, Pharmaceutical Society of Japan Award.
[Specialty] Synthetic organic chemistry

TCI Related Compounds of Contribution

Nitorbenzenesulfonyl Compounds

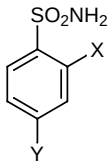
p.3-4

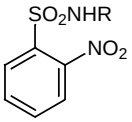
X=NO_2, Y=H: **NsCl** (**11a**)
25g, 500g [N0142]
X=H, Y=NO_2: ***p*NsCl** (**11b**)
5g, 25g [N0144]



p.4-5

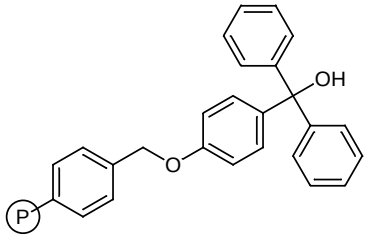
X=NO_2, Y=H: **$NsNH_2$** (**18**)
5g, 25g [N0705]
X=H, Y=NO_2 5g, 25g [N0697]





p.4-5

R=Boc (**19a**) 1g, 5g [B2303]
R=Alloc (**19b**) 5g [A1632]
R=Cbz (**19c**) 5g [C1757]

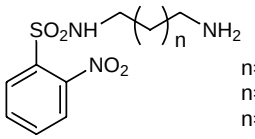


p.7 Resins (**41**)

4-Benzyloxytrityl Alcohol Resin, cross-linked with 1% DVB (200-400 mesh) (1.4-1.6 mmol/g) 1g [B2368]

p.6 Diamine derivatives

n=1	25ml, 500ml	[D0114]
n=2	25g, 500g	[D0239]
n=3	5ml, 25ml	[D0108]
n=4	25g, 500g	[D0095]
n=5	5g, 25g	[D0094]
n=6	25g	[D0107]
n=7	5g, 25g	[D0106]
n=8	25g	[D0085]
n=9	1g, 5g	[D2075]
n=10	25g, 500g	[D0091]



n=0	1g	[A1627]
n=1 (**27**)	1g	[A1628]
n=2 (**28**)	1g	[A1630]
n=3 •HCl	1g	[A1661]
n=4 •HCl	1g	[A1662]