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The five bromotryptophans

Review Article

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Summary. The five regioisomeric bromotryptophans (BrTrps) play an important role in the life of sponges and lower marine invertebrates. These bromo-amino acids, which are formed by post-translational modifications, are not found in nature in their free state, but rather are involved in more complex structures. Any of the BrTrps can be part of a peptide, a cyclic peptide, an indole alkaloid, an ergot alkaloid, a macrocycle and others. The present review covers the synthesis, physical and spectroscopic properties of the five BrTrps. It also describes the many exiting pharmacological and biological activities played by the BrTrps and by various secondary metabolites containing brominated tryptophan moieties. Of special interest are cyclic peptides containing the 2-BrTrp unit, which were isolated from marine sponges e.g. konbamide, orbiculamide A, the various keramamides, jaspamide eusynstyelamide and more. Important families of non-cyclic peptides containing the 6-BrTrp, include the styelins, the conotoxins, the cathelicidins and several constrained macrocyclic peptides. Many marine secondary BrTrp-containing, non-peptidic metabolites also display a remarkable spectrum of bioactivities, which can be harnessed for therapeutic and other purposes. Examples are: barettin, bromotryptanthrin, tetraacetyl clionamide, cyclocinamide A, clavicipitic acid, various brominated β-carbolines. In this review we have presented the various synthetic routes leading to the preparation of the five BrTrps and many of its derivatives. Also, we have introduced the reader to many synthetic routes leading to BrTrp-containing non-peptidic natural products. Although the functional role of the various compounds in the human body is only poorly understood, its effects were extensively studied. Almost all of these compounds exhibit important therapeutic properties e.g. antifungal, antimicrobial, antihelmintic, insecticidal ichthyotoxic and anticancer activity. In the present review attempts have been made to provide synopsis, synthesis and symbiosis of chemical and biological actions, which may provide future guidance and facilitate further research in this area.

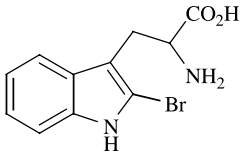
Keywords: Tryptophan – Bromotryptophan – Synthesis – Post-translational bromination – Cyclic peptides – Antimicrobial peptides

1. Introduction

The broad distribution of brominated natural products in the marine living arena arises presumably through the enzymatic action of a broad spectrum of bromo- and lactoperoxidases in marine organisms. Many compounds containing brominated indole rings were isolated and identified. Among them a large series of bromotryptophan (BrTrp) containing materials from lower marine invertebrates. The five known BrTrps are shown in Fig. 1. All the BrTrps were synthesized and their chemical and spectral data studied. In some cases only the racemic form or only a single enantiomer was characterized, in other cases the two optical isomers are known. These bromoamino acids are not found in nature in their free state, but rather are involved in more complex structures mainly from marine origin. All the BrTrps are formed by post-translational modification, that is, by bromination of tryptophan after translation (protein synthesis). This modification is essential for the particular functions of the non-proteinic or proteinic natural products.

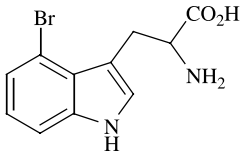
Any of the five BrTrps can be part of a peptide, a cyclic peptide, an indole alkaloid, an ergot alkaloid, a macrocycle and others. While all these BrTrp containing families show exiting pharmacological activities, the free amino acids themselves have only a limited number of potential biological uses. One exception is 5-BrTrp, which was found to be a relatively potent inhibitor of sickle-Hb polymerization.

Various cyclic peptides containing the 2-BrTrp units were isolated from marine sponges and their representatives are shown in Fig. 2. It is believed that these are antimicrobial peptides (AMPs), which form a first line of host defense against pathogens and are involved in innate immunity. The Konbamide from the Okinawan marine sponge *theonella* sp. (Kobayashi et al., 1991) has shown



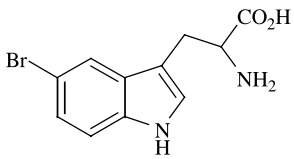
2-Bromotryptophan

(*S*)-form [89311-52-4]



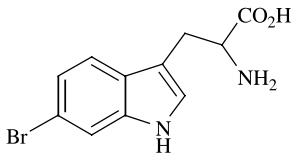
4-Bromotryptophan

(*S*)-form [52448-16-5]



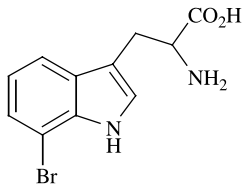
5-Bromotryptophan

(*R*)-form [93299-40-2]
(*S*)-form [25197-99-3]



6-Bromotryptophan

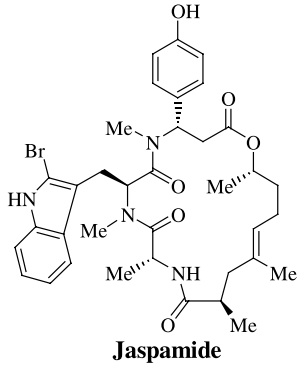
(*S*)-form [52448-17-6]



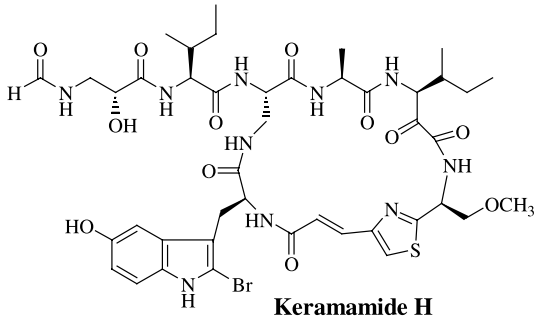
7-Bromotryptophan

(*S*)-form [75816-19-2]

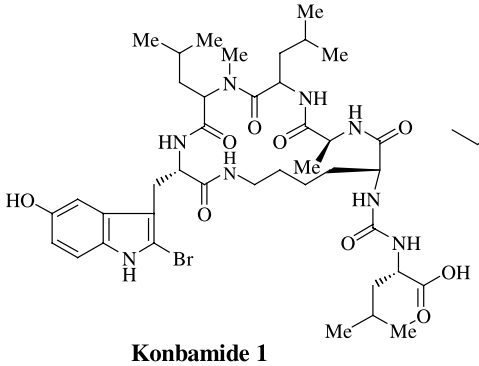
Fig. 1. The five bromotryptophans



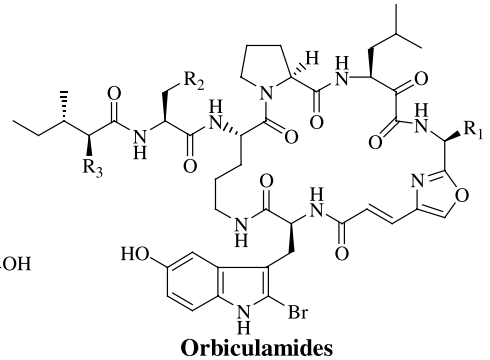
Jaspamide



Keramamide H



Konbamide 1



Orbiculamides

Fig. 2. Cyclic peptides containing the 2-BrTrp unit

to antagonize the effects of calmodulin in calmodulin-activated brain phosphodiesterase. Orbiculamide A was found to be cytotoxic against P388 murine leukemia cells. The various keramamides are potent inhibitors of the generation of the superoxide response of human neutrophiles elicited with fMLP. Jaspamide, a marine cyclodepsipeptide (Zabriskie et al., 1986), exhibits antifungal, antihelmintic, insecticidal and ichthyotoxic activity. The highly modified dimer peptide Eusynstyelamide from the ascidian *Eusynstyela misakiensis* is also a very bioreactive

compound (Swersey et al., 1994). In addition, these marine-sponge-derived unusual macrolides show actin-binding properties (Spector et al., 1999). Each of these drugs alters the distribution patterns of actin in a unique way, and has different biochemical properties and cellular effects.

Other non-cyclic peptides may vary in size from 7 to 41 amino acids with 1–5 disulfide bridges. Styelins, containing 6-BrTrp, are broad-spectrum antimicrobial peptides from the solitary tunicate *Styela clava.* Conotoxins are peptides also containing 6-BrTrp. They are paralytic poisons from Pacific cone snails that block the transmission of a nerve impulse from the nerve to the muscle at the neuromuscular junction. They can be used to modify the responses of tissues to pain stimuli and as drug leads for the development of novel therapeutics for the treatment of a range of neurological conditions. Another family of peptides is the cathelicidins, which show strong antimicrobial activity against both Gram-positive and Gram-negative aerobic and anaerobic bacteria. The constrained macrocyclic peptide analogs of TMC-95A show potential proteasome inhibition and the hexapeptide cyclocinamide A is cytotoxic. These are a few examples of the large library of BrTrp containing peptides from marine origin.

Many marine secondary non-peptidic metabolites also display a remarkable spectrum of bioactivities, which can be harnessed for therapeutic and other purposes. In this regard, BrTrp derivatives play a major role, both as vital intermediates and as final products. Examples are (Fig. 3): barettin, isolated from the marine sponge *Geodia barretti*, and found to be an excellent antifouling compound; the antibiotic bromotryptanthrin, a metabolic product of *Candida lipolytica;* tetraacetyl clionamide, from the sponge *Cliona celat*; cyclocinamide A isolated from the marine sponge *Psammocinia* sps.; clavicipitic acid an ergot alkaloid isolated from the *Claviceps* strain SD58 and from *Claviceps fusiformis*; *L*-6-bromohypaphorine, a new amino acid isolated from the sponge *Pachymatisma johnstoni* (Raverty et al., 1977); brominated β-carbolines isolated from the marine hydroid *A. pluma* (Aiello et al., 1987); the antibiotic alkaloid Eudistomin H isolated from the tunicate *Eudistoma olivaceum* (Shen and Baker, 1994).

Most of these natural products have the BrTrp moiety in their structure, which seems to be crucial to its biological activity. The list of BrTrp containing compounds and biological activity attributed is long and still growing. Design, synthesis and biological studies of such compounds require special techniques, procedures and bioas-

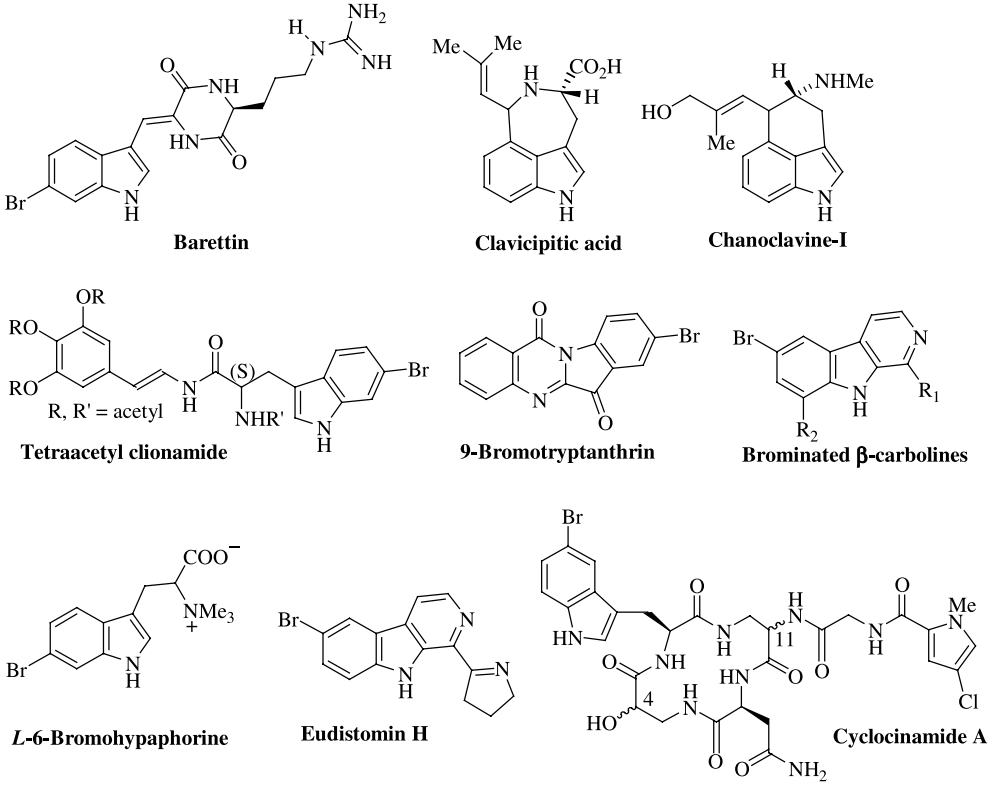


Fig. 3. Representative marine secondary non-peptidic metabolites

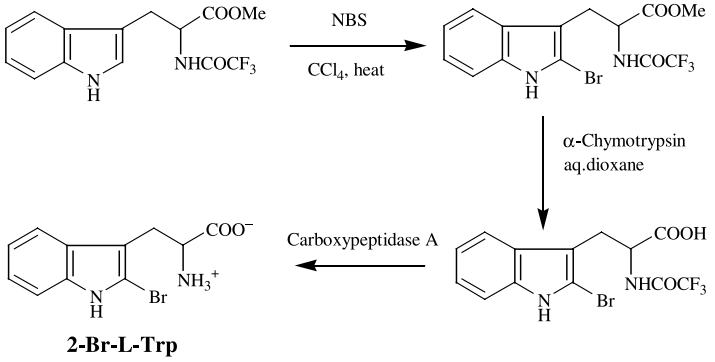
says. Thus, the accelerated growth in BrTrp materials requires a comprehensive and composite compilation. To date, no such a review is available. In the present review, attempts have been made to provide synopsis, synthesis and symbiosis of chemical and biological actions, which may provide future guidance and facilitate further research in this area.

2. Synthesis of bromotrypthophans

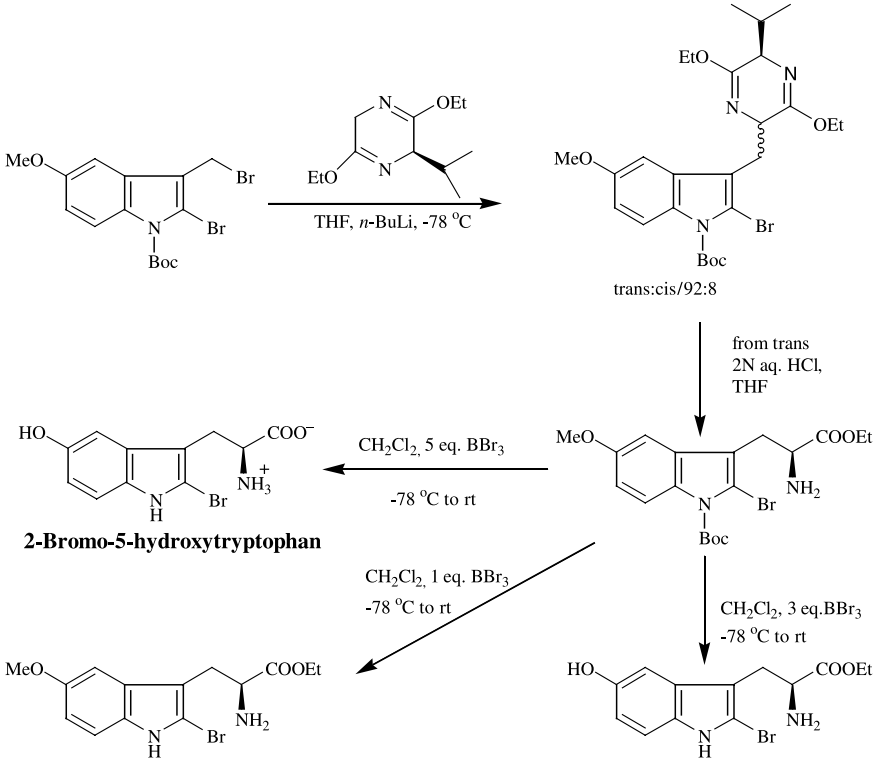
All of the five BrTrps were prepared by various synthetic methods. Some of the methods followed the classical amino acids syntheses, while others are stereoselective and include radical reactions, regiospecific bromination and enzymatic methods. The synthesized optically active BrTrps or derivatives are important building blocks for the synthesis of more complex natural products.

2.1. Synthesis of 2-BrTrp

Free radical bromination of CF_3CO-Trp-OMe by N-bromosuccinimide in CCl_4 gave 83% of protected 2-BrTrp. α-Chymotrypsin-catalyzed hydrolysis in aqueous dioxane gave 60% of CF_3CO-2-BrTrp, which was deacylated by carboxypeptidase A to give 70% L-2-BrTrp (Phillips and Cohen, 1983, 1986) (Scheme 1).



Scheme 1. Synthesis of L-2-BrTrp (Phillips and Cohen, 1983, 1986)



Scheme 2. Synthesis of optically active 2-bromo-5-hydroxytryptophan (Zhang et al., 1995)

A highly stereoselective synthesis of optically active 2-bromo-5-hydroxytryptophan and derivatives as well as a 2-BrTrp analog, was achieved in three steps from 2-bromo-3-bromomethylindole derivative (Zhang et al., 1995). Stereospecificity of the method is due to the use of the Schollkopf chiral auxiliary and the generation of the pyrazine intermediate (Scheme 2).

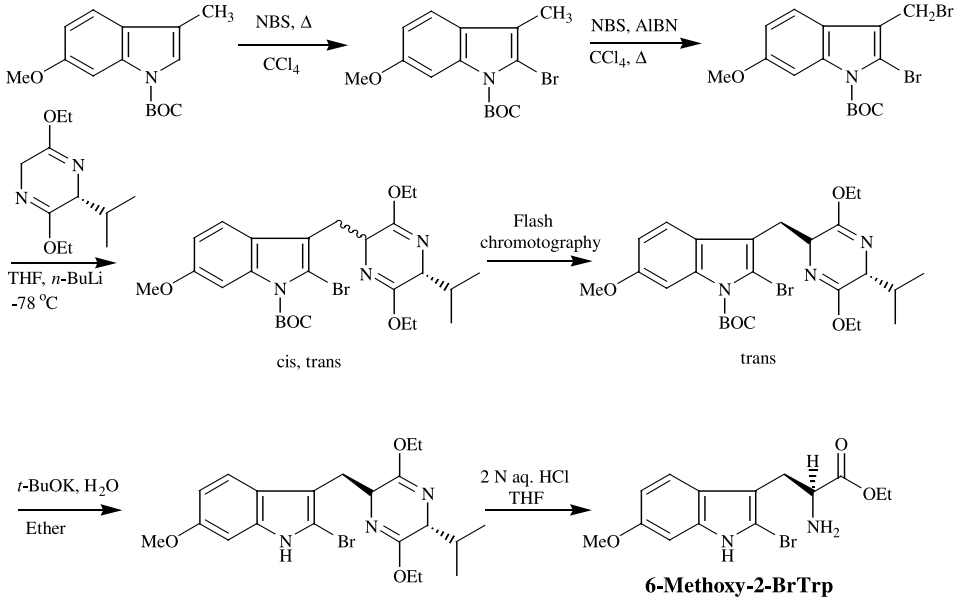
In a similar route, starting from 2-bromo-3-bromomethylindole, the D- or the L-2-BrTrp could be prepared *via* the auxiliary derived from L-valin.

A regiospecific bromination of substituted 3-methylindoles at either the C(3) alkyl moiety or the C(2) position was achieved *via* a free radical bromination or electrophilic process, respectively (Liu et al., 1997). The regiospecificity of the bromination could be controlled by variation of both the substituent and the N(1) protecting group on the indole ring. Optically active 2-BrTrp Et ester or its substituted derivatives could be prepared as in Scheme 2 in three steps using the 2-BrTrp compounds.

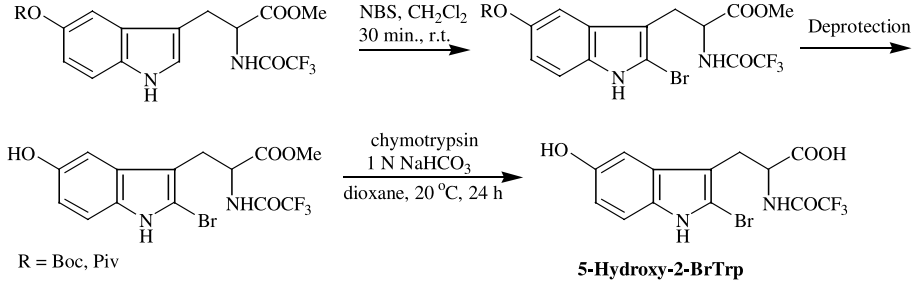
6-Methoxy-2-BrTrp ethyl ester, a potential intermediate for indole alkaloid synthesis, was prepared *via* the Fischer indole/Schollkopf protocol from 6-methoxy-3-methylindole including a regiospecific bromination process as a key step (Gan et al., 1997) (Scheme 3).

A similar synthesis of 5-hydroxy-2-BrTrp has been suggested (Schmidt et al., 1995). The synthesis includes the bromination of protected 5-hydroxytryptophan (Boc or pivaloyl) using NBS (Scheme 4).

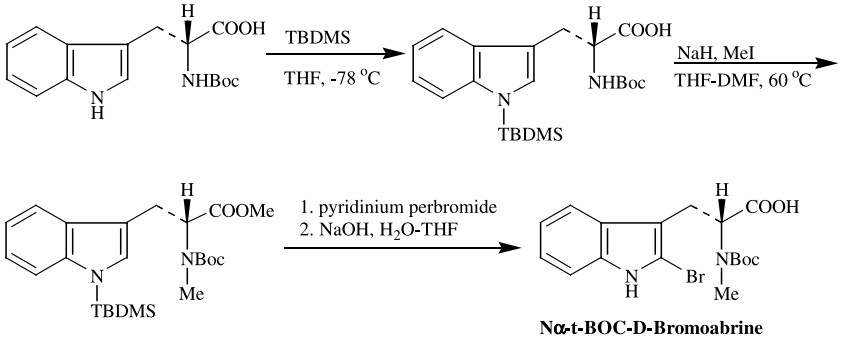
A synthesis of N^{α}-*t*-BOC-D-bromoabrine, a derivative of 2-BrTrp has also been performed (Grieco et al., 1988). The bromination of the N-methyl-tryptophan derivative was achieved using pyridinium bromide perbromide. The synthesis includes several steps starting from commercially available N^{α}-*t*-BOC-D-tryptophan and resulting



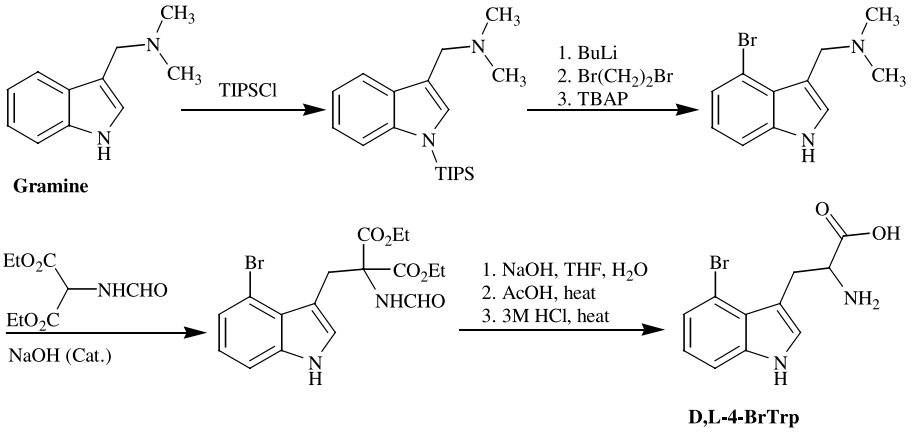
Scheme 3. Synthesis of 6-methoxy-2-BrTrp (Gan et al., 1997)



Scheme 4. Synthesis of protected 5-hydroxy-2-BrTrps (Schmidt et al., 1995)



Scheme 5. Synthesis of N^{α}-*t*-BOC-D-bromoabrine (Grieco et al., 1988)



Scheme 6. Synthesis of DL-4-BrTrp (Hurt et al., 1999)

with the methyl ester form, which is hydrolysed to yield the N^{α}-*t*-BOC-D-Bromoabrine (Scheme 5).

2.2. Synthesis of 4-BrTrp

On the way of developing an enantiospecific synthesis of (R)-4-amino-5-oxo-1,3,4,5-tetrahydrobenz[cd]indole, a synthesis of both racemic 4-BrTpt and derivatives of enantiomeric D-4-BrTrp were developed (Hurt et al., 1999). The racemic 4-BrTrp was made through the use of gramine as an inexpensive, readily available starting material (Scheme 6). Thus, the indole nitrogen of gramine was protected with TIPSCl, than reacted with BuLi to form the anion at the 4-position, which was quenched with 1,2-dibromoethane to give the 4-bromogramine derivative. Desilylation and treatment with diethyl N-formamidomalonate in presence of a catalytic amount of base, gave the indolyl malonate. Through a series of deprotection reactions, the free racemic 4-BrTrp was obtained in an overall yield of 72% from gramine.

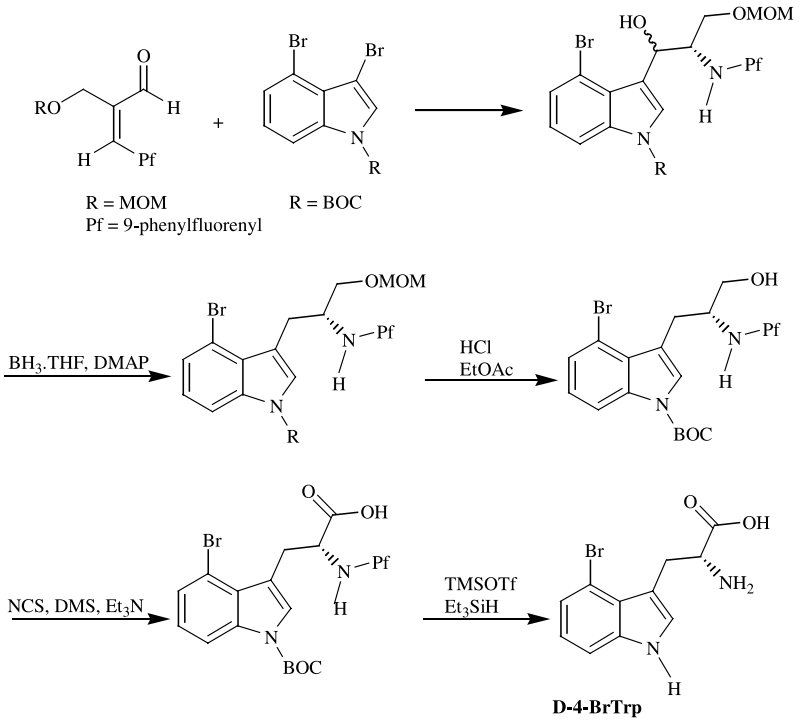
Enantiospecific synthesis of D-4-BrTrp was accomplished by the same scientists, *via* coupling of indolyllithium species with masked serinal (Scheme 7).

The key intermediate in the total synthesis of optically active clavicipitic acid is the optically active 4-BrTrp. It was prepared by the selective vinylation of 4-bromoindole with dehydroalanine in the presence of stoichiometric amounts of Pd(II) salt, followed by asymmetric reduction (Yokoyama, 1995; Yokoyama et al., 2004) (Scheme 8).

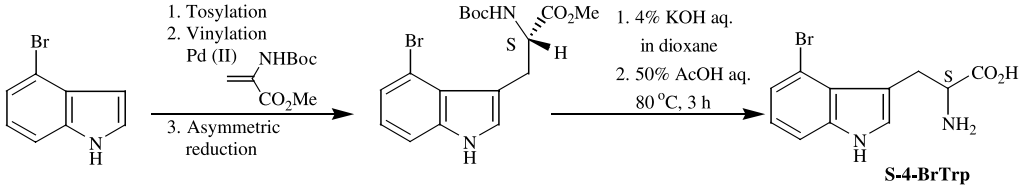
2.3. Synthesis of 5-BrTrp

Some initial attempts to synthesize 5-BrTrp and derivatives thereof were done already 50 years ago (Rydon and Tweddle, 1955; Harvey, 1959). However, a thorough and complete synthesis of both 5-bromo- and 7-bromo-L-tryptophans was achieved only in 1980 *via* the Fischer cyclization of the appropriate 4-bromophenylhydrazone of 4-acetamido-4,4-bis(ethoxycarbonyl)butanal (Allen et al., 1980) (Scheme 9).

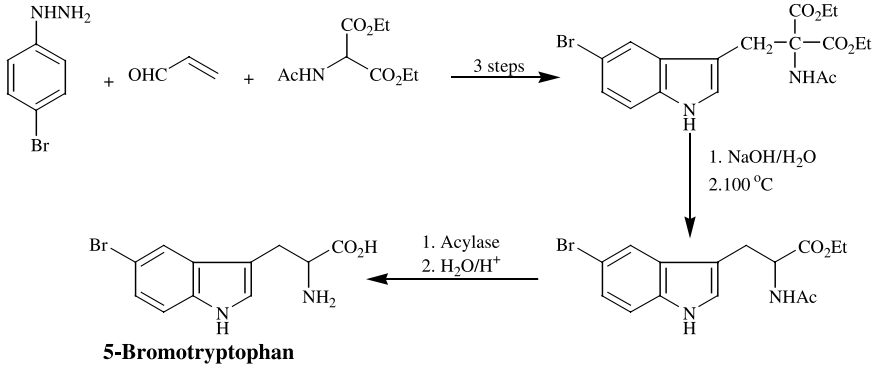
A convenient synthesis of DL-5-BrTrp and protected intermediates used for peptide synthesis, was performed (Prasitpan et al., 1990) by condensation of 5-bromoindole with $BrCH_2C(CO_2Et)$:NOH, followed by reduction (Zn) and saponification (Scheme 10).



Scheme 7. Synthesis of D-4-BrTrp (Hurt et al., 1999)



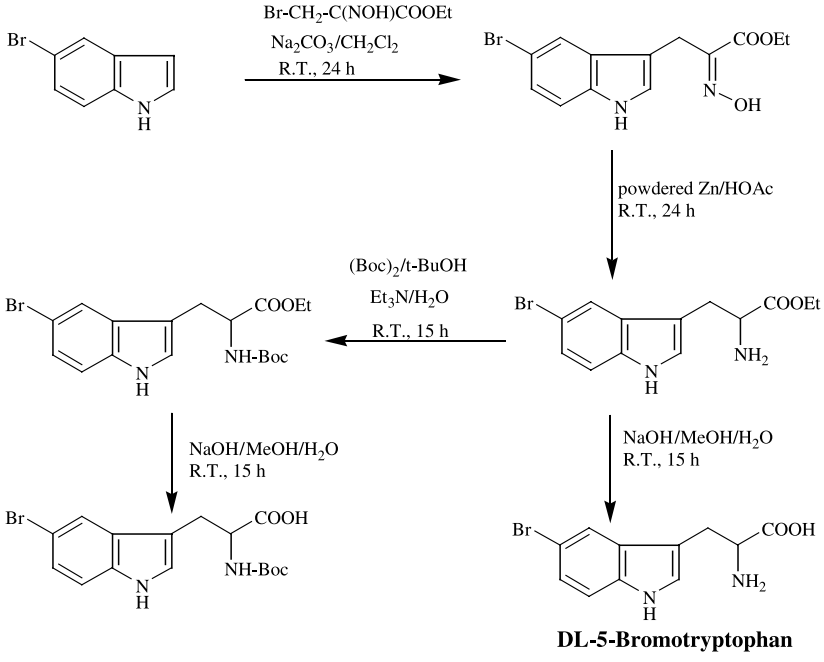
Scheme 8. Synthesis of optically active 4-BrTrp (Yokoyama et al., 1995a, 2004)



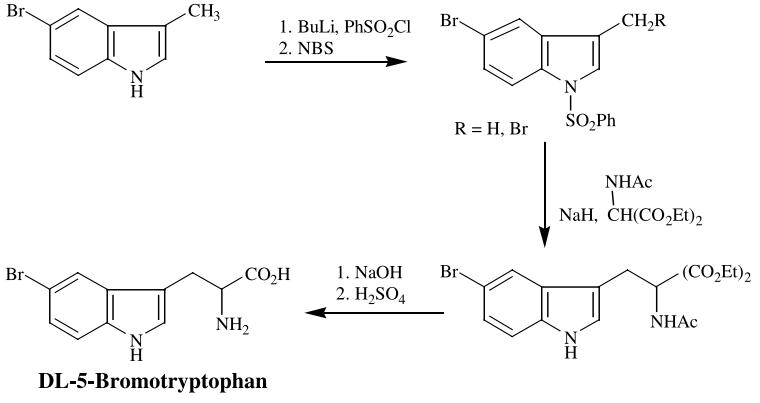
Scheme 9. Synthesis of 5-BrTrp (Allen et al., 1980)

Another modification of the synthesis of DL-5-BrTrp, from 5-bromo-3-methylindole was described (Nagarathnam and Johnson, 1993). The latter compound was treated with $PhSO_2Cl$ in the presence of BuLi to give the sulfonated indole, which was brominated (96%) with NBS in the presence of benzoyl peroxide in CCl_4. The resulting bromo derivative was treated with $AcNHCH(CO_2Et)_2$ in the presence of NaH to give the appropriate malonate (83%). Saponification with NaOH in aqueous EtOH and then hydrolyzes in refluxing 2N H_2SO_4 gave the 5-BrTrp in 76% yield (Scheme 11).

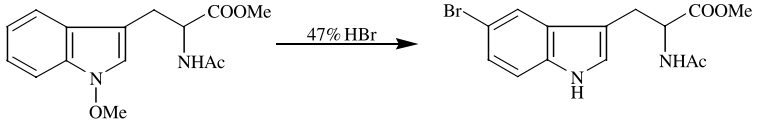
A novel methodology for introducing chlorine or bromine into the 5-position of tryptamines and tryptophans was introduced through the 1-hydroxy derivatives (Hasegawa et al.,



Scheme 10. Synthesis of 5-BrTrp (Prasitpan et al., 1990)



Scheme 11. Synthesis of DL-5-BrTrp (Nagarathnam and Johnson, 1993)



Scheme 12. Synthesis of DL-5-BrTrp (Hasegawa et al., 1999)

1999). The chemistry was applied to the synthesis of DL-5-BrTrp derivatives and DL-bromochelonin B (Scheme 12).

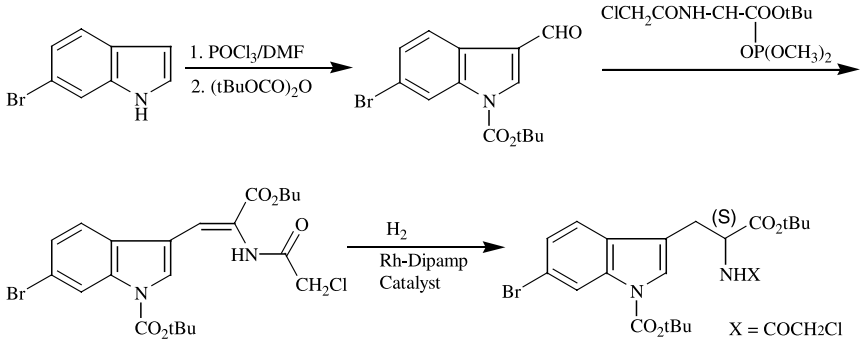
2.4. Synthesis of 6-BrTrp

In the course of the synthesis of hexaacetylcelenamid (see Scheme 21) the butyloxyderivative of S-6-BrTrp was prepared in enantioselective mode (Schmidt and Wild, 1985) (Scheme 13). The process started with Vilsmeier reaction, followed by Horner condensation and finally reduction with Rhodium-Dipamp catalyst.

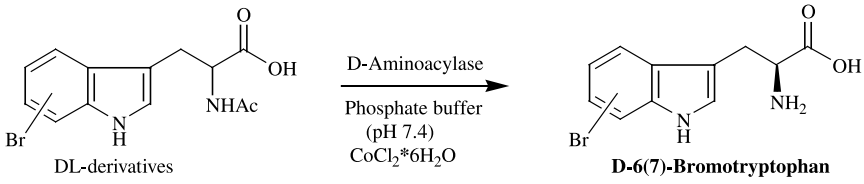
The two unusual amino acids, 6 and 7-bromo-D-tryptophan are important intermediates in the synthesis of the right hand segments of kistamycin A and chloropeptin. These two amino acids and their derivatives were conveniently constructed using optical enzymatic resolution starting from N-acetyl-6-bromo- and N-acetyl-7-bromo-DL-tryptophan and using D-aminoacylase from *Achromobacter xylosoxydans aubap. xylosoxydans* (Konda-Yamada et al., 2002) (Scheme 14).

2.5. Synthesis of 7-BrTrp

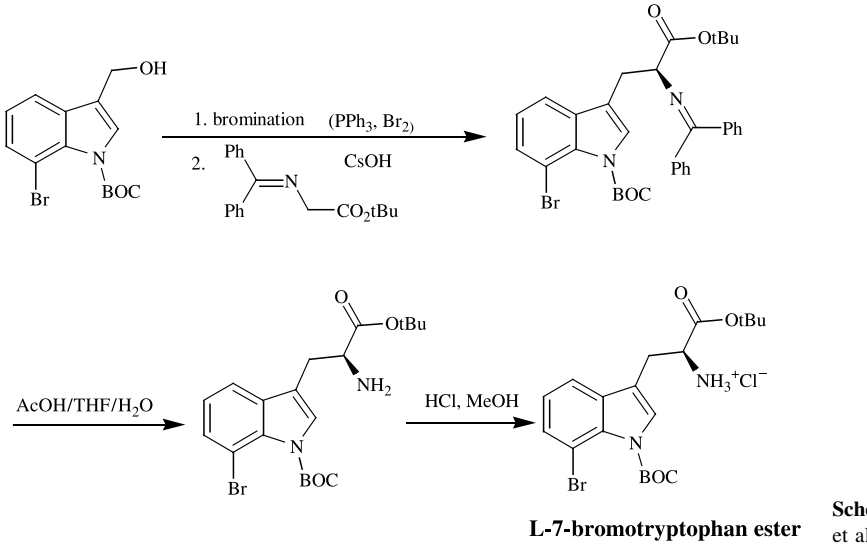
Enantiomeric 7-BrTrps and derivatives are important intermediates for the synthesis of Kistamycin A and Chloropeptin. L-7-BrTrp could be prepared by a Fischer



Scheme 13. Synthesis of protected S-6-BrTrp (Schmidt and Wild, 1985)



Scheme 14. Enzymatic synthesis of D-6(7)-BrTrp (Konda-Yamada et al., 2002)



Scheme 15. Synthesis of L-7-BrTrp ester (Berthelot et al., 2003)

cyclization of the appropriate bromo-phenylhydrazone of 4-acetamido-4,4-bis(ethoxycarbonyl)butanal in a similar methodology described above for the 5-BrTrp (Allen et al., 1980) (see Scheme 9 for the synthesis of 6-BrTrp). D-7-BrTrp and its protected derivatives were conveniently synthesized by optical enzymic resolution from N-acetyl-DL-7-BrTrp using D-aminoacylase (Konda-Yamada et al., 2002) (see Scheme 14 for the synthesis of 6-Br-D-Trp).

An enantioselective preparation of L-7-BrTrp ester was carried out, using a Corey-O'Donnell alkylation protocol (Berthelot et al., 2003) (Scheme 15). The glycine benzophenone imine was achieved in good overall yield with very high enantiomeric excess (>85%) on a multigram scale.

3. Some physical, structural and biological features of the bromotryptophans

3.1. UV-vis

UV spectra of halogenated indoles and tryptophan in solution show a distinctive red-shift in their λ_{max} values for the band corresponding to their π–π^* transition (Shinnar et al., 2005). Time-dependent density functional theory (TDDFT) calculations revealed an increasing trend in the energy of the HOMO-LUMO transitions: 5-<6-<7-<4-bromoindole regioisomers. These energy differences, arising from the relative destabilization of the HOMO-LUMO transitions, parallel the experimental red shifts. In solution, the largest red shift occurs for the 5-regioisomer ($\Delta\lambda_{max} = 9$ nm), fol-

Table 1. Some physical properties of BrTrps

Name	Form	Mp (°C)	$[\alpha]_D^{20}$
2-BrTrp	S	195–200	+1.2 (in H_2O) +20.8 (MeOH)
4-BrTrp	R	288–290	+27.6 (1 M HCl)
	RS	287–289	
5-BrTrp	RS	261–262	
	S	288–291 (dec.)	−28.5 (AcOH) +31.8 (1 M HCl)
	R	289–290	+27.1 (AcOH)
6-BrTrp	R	232–236	+22.4 (MeOH)
	S	125–127	−18.6 (MeOH)
7-BrTrp	R	244–258	+11.4 (MeOH)
	S	220–222	−24.0 (MeOH)

lowed by the 6-regioisomer ($\Delta\lambda_{max} = 6$ nm). The red shift shows only a very slight dependence on % organic co-solvent. These red shifts are useful in identifying the different regioisomers found in marine peptides.

3.2. Phosphorescence

Tryptophan phosphorescence provides a long-lived optical signal, whose lifetime and quantum yield are sensitive to the local environment of the protein. The room temperature phosphorescence of 5-BrTrp embedded in sucrose glasses was studied (Mccaul and Ludescher, 1998). The absorption of this analog was markedly red-shifted with respect to tryptophan and the phosphorescence emission spectrum was also red-shifted with maxima at 452 nm (as compared with 442 nm for tryptophan). The emission intensity and relative quantum yield (0.022) of the 5-BrTrp was much lower compared to that of tryptophan. 5-BrTrp also exhibited complex decay behavior, which required several exponentials for an adequate fit. The average lifetime was significantly lower (1.03 ms) than that of tryptophan (2109 ms).

In order to assess the potential of various tryptophan analogs including 5-BrTrp and 6-fluorotryptophan to act as structure-conserving substitutes for tryptophan, their fluorescence characteristics were studied using two novel fluorescence spectroscopic techniques for a wide range of solvent polarities (Lotte et al., 2004). Two-dimensional mapping of all emission and all fluorescence spectra, using excitation-emission spectroscopy (EES), has been used to determine quantum yields, positions of emission maxima, full widths at half maximum (FWHMs), as well as Stokes' shifts. Additional fluorescence lifetimes were obtained from time-resolved experiments using a picosecond laser system and compared with the data acquired from the static setup. Phosphorescence and optically detected magnetic resonance (ODMR) spectra of tryptophan, 5-BrTrp and of 4-,5-,6-methyltryptopan; 4-,5-,6-fluorotryptophan, were compared with those of complexes formed with tryptophan-free tryptophan apo-repressor from *Escherichia coli* (W19, 99F). *E. coli* was found to bind tryptophan and each analogue, except 4-fluorotryptophan, with an estimated K_D smaller or equal to 30 μM (relatively high binding affinity). Triplet-state spectroscopic and kinetic effects that accompany binding at the co-repressor site were also reported (Ozarowski et al., 1998).

3.3. Mass spectrum and X-ray crystallography

Analysis of BrTrp modifications was performed by high-resolution, high-accuracy precursor ion scanning utilizing fragment ions with mass-deficient mass tags (Steen and Mann, 2002). Its immonium ion was described as 'two fragment ions' characteristic for tryptophan-brominated peptides. The "reporter" ion of brominated tryptophan peptides is highly mass deficient due to the presence of bromine, thereby allowing the selective detection of these species and the distinction from other dipeptidic α-, β-, and γ-fragment ions. High-resolution, high-accuracy precursor ion scanning enabled the identification of brominated tryptophan species and the directed analysis of species carrying these modifications in a highly complex *Conus textile* conotoxin mixture. This method enabled the characterization of one novel *C. textile* conotoxin containing a BrTrp residue.

The tryptophan RNA-binding attenuation protein (TRAP) of *Bacillus subtilis* was crystallized and examined by crystallography using X-ray synchrotron radiation diffraction data (Antson et al., 1994). Crystals of a potential heavy-atom derivative of TRAP complexed with L-5-BrTrp grow in the same space group with similar cell dimensions. The results obtained lay the groundwork for determination of the TRAP three-dimensional structure by isomorphous replacement.

3.4. Biological activities

While many derivatives and peptides containing BrTrp show profound and critical biological and pharmacological activities, the free amino acids themselves have only limited number of potential biological uses. One exception is 5-BrTrp, which was found to be a relatively potent inhibitor of sickle-Hb polymerization (see Section 5.1). Of all the five amino acids, only 5-BrTrp was tested for biological activity.

DL-5-BrTrp was tested in vitro for antiviral effect against Columbia SK, lymphocytic choriomeningitis, vaccinia, or adeno type 12 viruses (Furusawa et al., 1964). Some effects were witnessed but only against a deoxyribonucleic acid type (vaccinia) and a ribonucleic acid type (Columbia SK) virus. In another experiment DL-5-BrTrp was tested as possible inhibitor of serotonin biosynthesis (De Ropp and Furst, 1966). When injected intraperitoneally in mice at levels up to 1000 mg/kg, it markedly lowered random motor activity, noticeable 5 min after injection and persisting for over 90 minutes. *DL*-5-BrTrp was an effective agent at increasing the threshold on self-stimulation of rats having electrodes implanted in the medial forebrain bundle (Gibson et al., 1970).

5-Halotryptophans were found to act as brain tyrosine hydroxylase inhibitors (McGeer et al., 1967). Substitution at the 5-position of the indole ring is apparently superior to substitution at either the 4 or 6 positions for the inhibition of the brain enzyme. The decreasing order of activity of the series was found to be: iodo-> bromo-> chloro-> fluorotryptophans in rat brain homogenates. DL-5-BrTrp possessed high activity, producing 50% inhibition at 3×10^{-6} M. It was considerably less active against beef adrenal tyrosine hydroxylase than against the brain enzyme. The aliphatic side chain is apparently important for the high inhibitory activity against brain tyrosine hydroxylase, since 5-bromoindole, 5-bromogramine and di-Et-α-formamido-α-(5-bromo-3-indolylmethyl) malonate, had no appreciable inhibitory activity.

4. Various roles of bromotrypthophans in the synthesis of natural occurring non-peptidic compounds

Many marine secondary metabolites display a remarkable spectrum of bioactivities, which can be harnessed for therapeutic and other purposes. Developing synthetic methods for their preparation is of much importance, as it furnishes the basis for large-scale production of such materials. In this regard, BrTrp derivatives are playing a major role, both as vital intermediates and as final products.

Thus, the secondary metabolite, Barettin (cyclo[6-bromo-8-entryptophan] arginine), isolated from the marine sponge *Geodia barretti*, is known to inhibit the settlement and/or attachment of marine sessile organisms (e.g. invertebrate larvae). The latter are causing biofouling on ship hulls and on static constructions, such as offshore oil rigs and pipelines. The development of an efficient synthetic method for a large-scale production of Barettin, or similar antifouling compounds from marine origin, will allow the substitution of the currently in use heavy metal-based coatings, which are facing bans due to their environmental hazard (Sjogren et al., 2004).

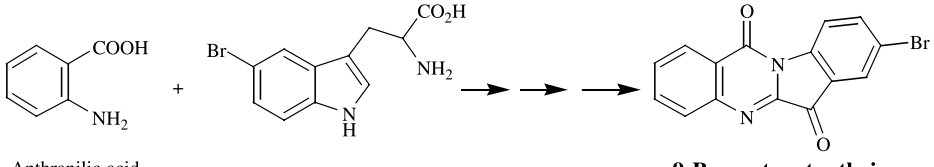
Synthetic methods involving BrTrp derivatives have been developed, mimicking the production of bromine-containing peptides of therapeutic value. Thus, a synthetic route was developed for the antibiotic bromotryptanthrin, a metabolic product of *Candida lipolytica*. D-4-bromotryptophan served as an intermediate in the enantiospecific synthetic route of the tricyclic core of many tetracyclic ergot alkaloids, Tetraacetyl clionamide, a natural product of the sponge *Cliona celata*, cyclocinamide A, an hexapeptide isolated from the marine sponge *Psammocinia* sps., clavicipitic acid and others were recently synthesized. In this section we will present the methodology used in these syntheses.

Tritiation of tryptophan residues could be successfully achieved through the corresponding 5-bromoderivatives. This method was used to synthesize tritium-labelled biologically active analogs of somatostatin (Allen et al., 1980).

4.1. Synthesis of alkaloids

Candida lipolytica can bio-synthesize the antibiotic tryptanthrin from one mole of tryptophan and one mole anthranilic acid. When supplying bromotryptophan and anthranilic acid, the bromo derivatives of tryptanthrin was formed and identified (Fiedler et al., 1976). The 9-bromo compounds turned out to be especially effective antibiotics (Scheme 16).

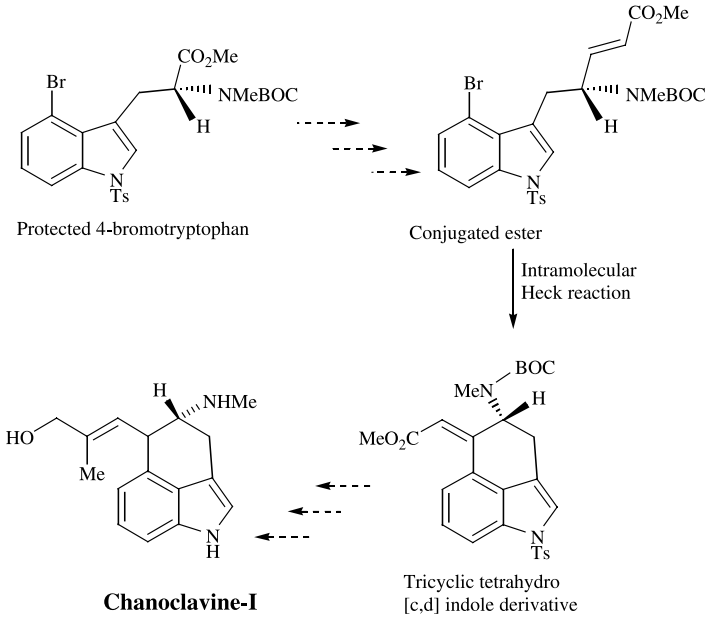
The total synthesis of the optically active chanoclavine-I, an ergot alkaloid, was accomplished using palladium-



Anthranilic acid

9-Bromotryptanthrin

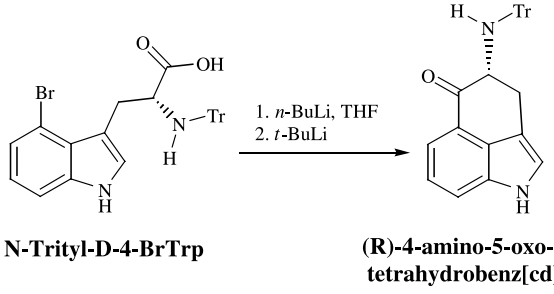
Scheme 16. Biosynthesis of 9-bromotryptanthrin (Fiedler et al., 1976)



Scheme 17. Synthesis of chanoclavine-I (Yokoyama et al., 1996)

catalyzed intramolecular cyclization (Heck reaction) as a key step (Yokoyama et al., 1996, 1996a). The conjugated ester was obtained in several steps from an optically active 4-BrTrp and its cyclization proceeded smoothly without racemization to give the key intermediate, tricyclic tetrahydrobenz[c,d]indole derivative, in high yield (Scheme 17).

A new strategy was described for the enantiospecific synthesis of (R)-4-amino-5-oxo-1,3,4,5-tetrahydrobenz[cd] indole (Hurt et al., 1999). This compound is an advanced intermediate, which contains the tricyclic core of many of the tetracyclic pharmacological active ergot alkaloids. The method involves the use of D-4-BrTrp. The α-amino position was protected with an N-trityl group, ensuring the enantiomeric integrity of this position during the ensuing organometallic cyclization reaction. Stabilization of the tricycle was accomplished by protecting the indole nitrogen with a BOC group, or by reducing the α-amino ketone to the corresponding β-amino alcohol (Scheme 18).



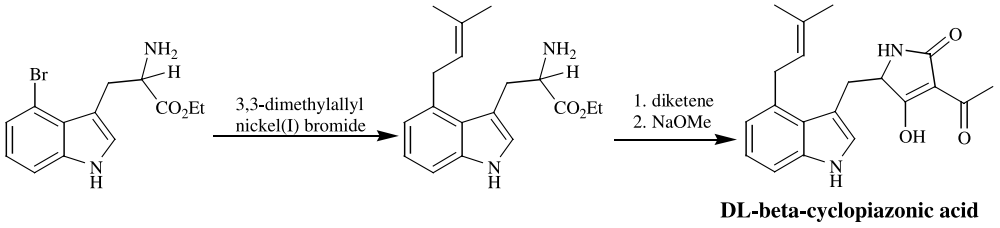
Scheme 18. Synthesis of (*R*)-4-amino-5-oxo-1,3,4,5-tetrahydrobenz[cd] indole

4.2. Synthesis of various complex indole derivatives

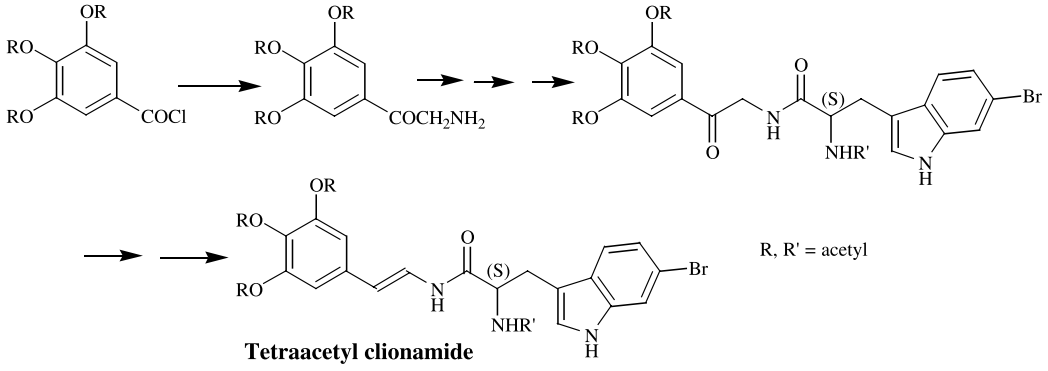
β-Cyclopiazonic acid is the direct precursor of α-cyclopiazonic acid, a toxic metabolite of *Pencillium cyclopium* Westling. The synthetic procedure for *DL*-β-cyclopiazonic acid involves the reaction developed by Pleninger for the introduction of an allyl side chain into the 4-position of indoles (Plieninger and Sirowej, 1971). Thus 4-BrTrp Et ester was reacted with π(3,3-dimethylallyl) nickel bromide to give 75% 4-(3,3-dimethylallyl)-tryptophan ethyl ester and 14% tryptophan Et ester. The former was converted into *DL*-β-cyclopiazonic acid by the reaction with diketene, followed by treatment with base (Holzapfel and Gildenhuys, 1977) (Scheme 19).

Clionamide, a 6-bromotryptophan derivative, was isolated from the sponge *Cliona celata* as the tetraacetate and its structure was determined by chemical and spectral data (Andersen, 1978).

Tetraacetyl clionamide was synthesized from 3,4,5-$(AcO)_3C_6H_2COCl$ (triacetyl gallic acid chloride) in 10 steps with a 45% yield (Schmidt et al., 1982, Schmidt and Wild, 1985). The triacetyl gallic acid chloride was reacted successively with diazomethane, HCl and sodium azide to yield azido ketone which was hydrogenated to the amino ketone. Acylation with S-Boc-6-bromotryptophan pentafluorophenyl ester was done, followed by the reduction with $NaBH_3CN$. Oxidative elim-



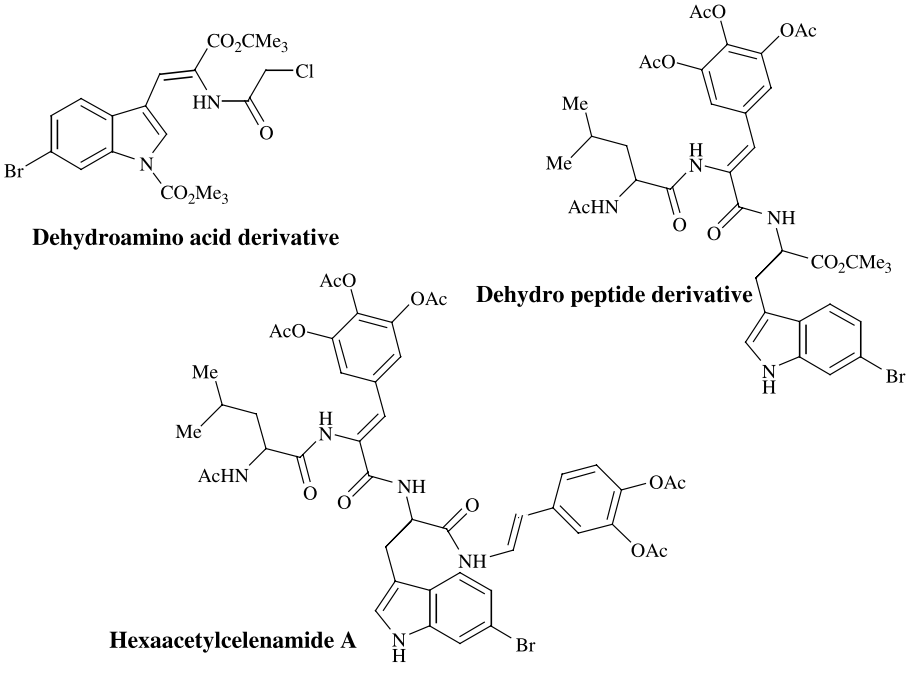
Scheme 19. Synthesis of *DL*-β-cyclopiazonic acid (Holzapfel and Gildenhuys, 1977)



Scheme 20. Synthesis of tetraacetyl clionamide

ination reaction (with *p*-nitrophenyl selenocyanate and tributylphosphine) yielded triacetyl-Boc-clionamide, which was deprotected and acetylated to yield tetraacetyl clionamide (Scheme 20).

The total synthesis of the linear peptide alkaloid hexaacetylcelenamide A was designed and executed along similar lines. The key steps are two condensation reactions with α-dialkylphosphoryl amino acid to obtain the dehy-



Scheme 21. Hexaacetylcelenamide A and its two synthetic intermediates (Schmidt and Wild, 1985)

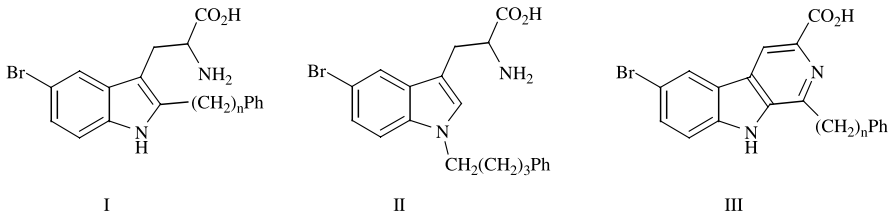
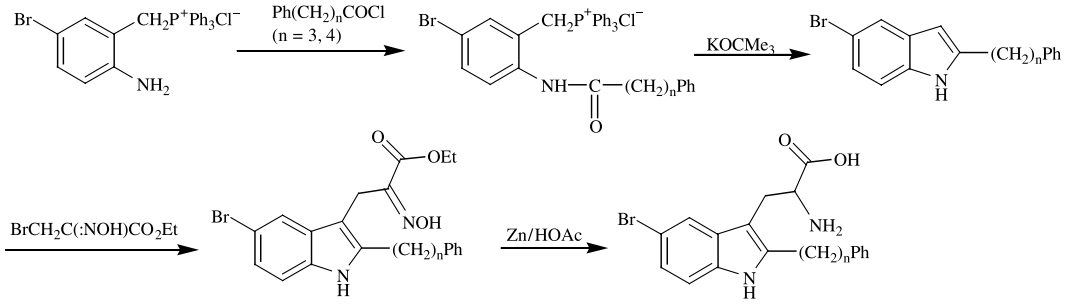


Fig. 4. C-1 Phenylalkyl-substituted 3,4-dihydro-β-carbolines



Scheme 22. Synthesis of 5-BrTrp derivatives, potential inhibitors of sickle cell Hb gelation (Prasitpan et al., 1992)

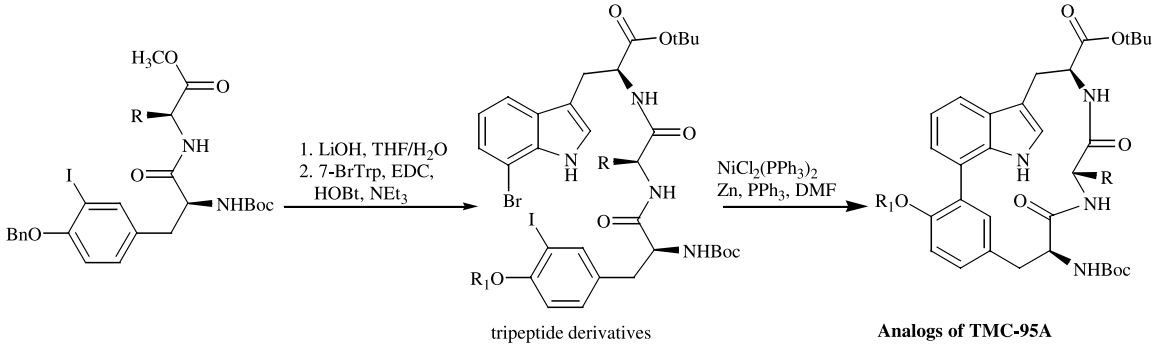
droamino acid and dehydro peptide derivative (Schmidt and Wild, 1985) (Scheme 21).

5-Bromotryptophan derivatives of type I, II and III (C-1 phenylalkyl-substituted 3,4-dihydro-β-carbolines) (Fig. 4) were prepared as potential inhibitors of sickle cell Hb gelation (Prasitpan et al., 1992). The syntheses begin from bromoanilines, which are acylated with $Ph(CH_2)_nCOCl$ ($n = 3$, 4) to give the corresponding N-acyl derivatives. Cyclization yields the corresponding bromoindoles. Alkylation with $BrCH_2C(:NOH)CO_2Et$ gave the oximes, which were reduced by Zn in HOAc and saponified to the desired derivatives (Scheme 22). None of the compounds were more potent than 5-bromotryptophan itself as inhibitors of sickle cell Hb gelation.

In the synthesis of constrained macrocyclic peptide analogs of TMC-95A as potential proteasome inhibitors, the key step involves a Ni(0)-mediated macrocyclization of the tripeptides bearing brominated indole side chains for the formation of the biaryl junction (Berthelot et al., 2003) (Scheme 23).

4.3. Barettin

Barettin, a pharmacologically active indole alkaloid, was isolated in 1986 from the cold water sponge *Geodia barrette* (Lidgren et al., 1986). The compound was isolated through its ability to inhibit the settlement of cyprid larvae of the barnacle Balanusimprovisus. Its structure has been the subject of debates for several years. The originally proposed structure, the diketopiperazin 1 was disproved a year later by an independent total synthesis of both **1** and its E-isomer **2** (Lieberknecht and Griesser, 1987). In 2002 a German



Scheme 23. Synthesis of TMC-95 analogs (Berthelot et al., 2003)

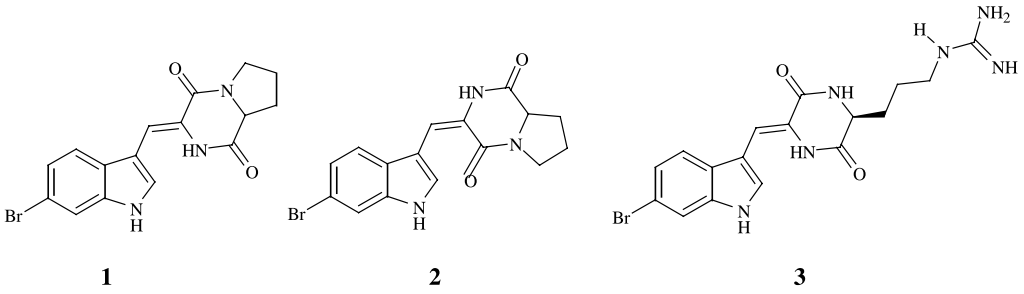
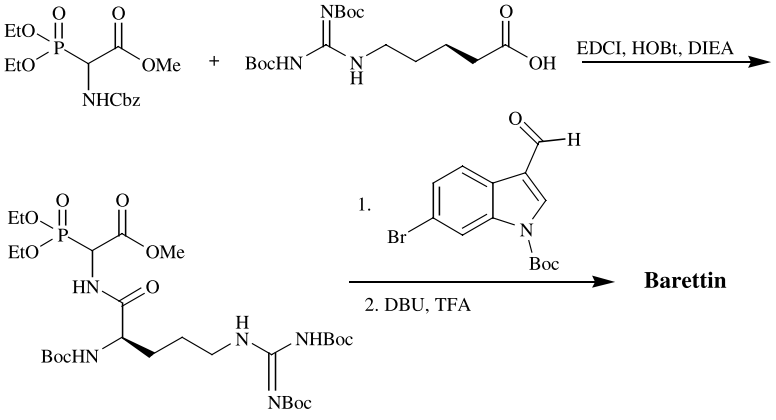


Fig. 5. Three proposed structures of Barettin



Scheme 24. Total synthesis of Z-Barettin (Johnson et al., 2004)

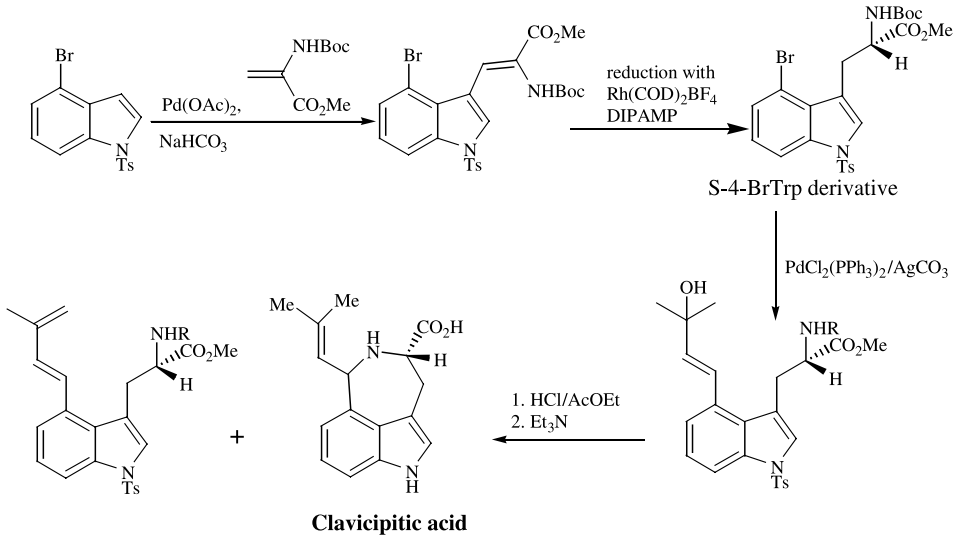
group isolated a diketopiperazine produced by the marine sponge *G. baretti* collected in Norway (Solter et al., 2002). Structure elucidation proved it to be a condensation product of 6-BrTrp and arginine, i.e. compound **3**. Based on the comparison of spectral data, it was stated that **3** represent the actual structure of Barettin (Fig. 5).

These findings were later confirmed (Johnson et al., 2004) by reinvestigation of the isolated material, combined with a total synthesis of **3**. It was synthesized *via* a Horner-Wadsworth-Emmons type reaction from 6-bromoindole-3-carboxaldehyde to introduce the dehydro-functionality. Subsequent deprotection and cyclization afforded the natural product in the Z-conformation (Scheme 24).

The potent antifouling effects of a Z/E mixture of barettin isolated from the marine sponge *Geodia barrette* was studied by the same research team (Sjogren et al., 2004). The activities of this brominated diketopiperazine-like cyclic dipeptide are in the range of antifouling agents in use today. However, contrary to today's antifouling agents, the effects of barettin are nontoxic and reversible. Synthetic analogues including DL-5-BrTrp, and DL-6-BrTrp, were tested for possible structure-activity relationships. None of these compounds showed any effect at a concentration of 10 μM. It was hypothesized that barettin is part of the sponge's chemical defense to deter fouling organisms. This theory is supported by the fact that barettin is found in water exposed to living specimens of *G. barretti* in concentrations that completely inhibit barnacles from settling.

4.4. Clavicipitic acid

Clavicipitic acid is an ergot alkaloid that has been isolated from the *Claviceps* strain SD58 and from *Claviceps fusiformis* as a mixture of diastereomers. The first total synthesis of optically active clavicipitic acid used optically active 4-BrTrp as the key intermediate (Yokoyama et al., 1995a). Palladium-mediated chemoselective vinylation of 4-bromo-1-tosylindole gave the 4-bromodehydrotryptophan, which was converted to optically active 4-BrTrp derivative by asymmetric reduction in the presence of DIPAMP-rhodium complex. Vinylation with 2-methyl-3-buten-2-ol at the C4-position in the presence of a catalytic amount of $PdCl_2(PPh_3)_2$ gave (S)-N-tert-butoxycarbonyl-4-(3-hydroxy-3-methyl-1-buten-1-yl)-1-tosyltryptophan, which is regarded as a synthetic equivalent of the biosynthetic key intermediate 4-(γ,γ-dimethylallyl)tryptophan. The cyclization occurred simultaneously with the loss of the tert-butoxycarbonyl group by treatment with HCl-saturated AcOEt, to give the cyclized product Na-tosyl-



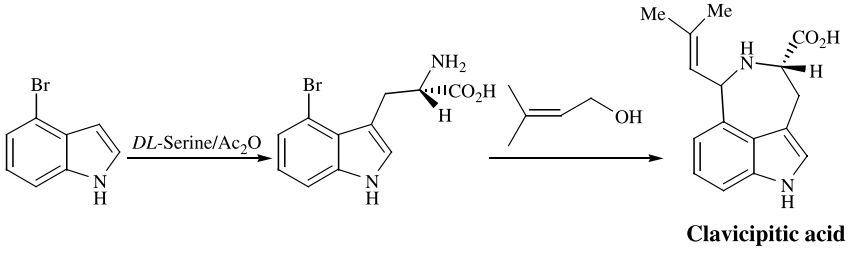
Scheme 25. Synthesis of clavicipitic acid (Yokoyama et al., 1995a)

clavicipitic acid methyl ester. The detosylation of each isomer with Mg/MeOH followed by hydrolysis with KOH/MeOH gave optically pure clavicipitic acid (*trans* or *cis*) (Scheme 25).

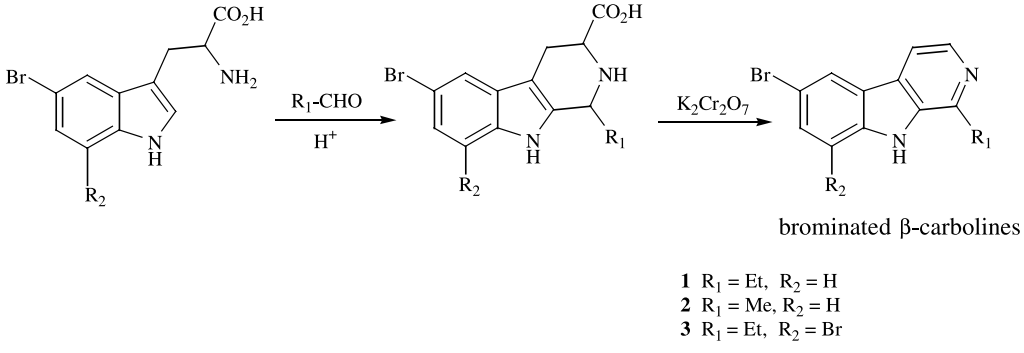
Several years later, the same authors synthesized optically active clavicipitic acid by a three-step biomimetic sequence from 4-bromoindole (Yokoyama et al., 1999, 2001, 2004). Thus, reacting 4-bromoindole with *DL*-serine in the presence of Ac_2O followed by enzymatic kinetic resolution gave (S)-4-bromotryptophan. The Heck reaction of (S)-4-BrTrp with 1,1-dimethylallyl alcohol in aqueous media gave clavicipitic acid (Scheme 26). This quite short synthesis was made possible by omitting the protection and deprotection steps in the synthetic route.

4.5. Brominated β-carbolines

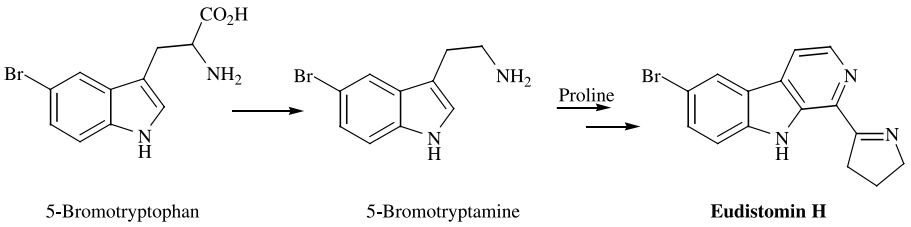
The interest in brominated β-carbolines grew lately because of their antiviral and antimicrobial activities. Three brominated β-carbolines (**1**–**3**) were isolated from



Scheme 26. Synthesis of optically active clavicipitic acid (Yokoyama et al., 1999)



Scheme 27. Synthesis of brominated β-carbolines



Scheme 28. Synthesis of eudistomin H

the lipophylic extract of the marine hydroid *Aglaphenia pluma* and their structures were determined on the basis of spectral data including 2-dimensional ^{1}H and ^{13}C shift correlation (direct and long-range) NMR spectroscopy (Aiello et al., 1987). The syntheses of **1**–**3**, starting from the appropriate brominated tryptophan derivatives, were also performed (Scheme 27).

A large series of carboline derivatives were similarly prepared in a 2-step synthesis from *p*-anisaldehyde and 5-BrTrp. They were useful in the inhibition of angiogenesis (Moon et al., 2005) and tested in assay evaluating their effect on hypoxia-inducible endogenous VEGF expression.

Eudistomins are a series of amino acid derived β-carboline antibiotics produced by the tunicate *Eudistoma olivaceum*. Tunicate metabolites have extracted considerable attention because of their pharmacological activities. The antiviral oxathiazepine-bearing eudistomins, the didemnins, and the ecteinascidins are potent and selective cytotoxins currently under clinical or preclinical developments. The biosynthetic origin of the antibiotic alkaloid Eudistomin H is believed to be 5-bromotryptamine and 5-BrTrp as intermediates (Shen and Baker, 1994). Indeed, both these amino acid derivatives served efficiently as precursors to its preparation (Scheme 28).

5. Natural peptides including bromotryptophans

Many of the common amino acids can be modified post-translationally to yield a set of additional amino acids that contribute to the function of mature proteins. These secondary amino acids, together with the 22 primary amino acids comprise the set of proteinogenic amino acids. In 1997 it was established (Craig et al., 1997) that the *L*-6-BrTrp is a secondary amino acid, present in two peptides purified from the venoms of two *Conus* species. Since then, the residue *L*-6-BrTrp was identified in the sequence of a heptapeptide isolated from *Conus imperialis*, a worm-hunting cone and in a 33-amino acid peptide from *Conus radiatus*, which induces a sleep-like state in mice (the bromosleeper peptide). It has been speculated that the tryptophan residues in the above-mentioned peptides were brominated in vivo in a eukaryotic system. Both the styelins isolated from solitary ascidian and the cathelicidins isolated from hagfish organ systems, contain several post-translationally modified amino acids, including also the 6-BrTrp.

It was found that the venom of predatory marine snails is a rich source of natural products that act on specific receptors and ion channels within the mammalian nervous system. 5-BrTrp and several of its peptides (e.g. 5-BrTrp-Gly-5-BrTrp-NH_2) were found to be the best polymerization inhibitor of deoxy sickle cell hemoglobin S (Hb S) within the erythrocytes, which is the primary molecular event linked to sickle cell disease.

5.1. Cathelicidins

In searching for natural host defense molecules, the Atlantic hagfish, *Myxine glutinosa* was examined (Shinnar et al., 1996). Preliminary screening of hagfish organ systems indicated that the intestinal tissue was rich in antimicrobial activity. From intestine extracts, a family of antimicrobial peptides (HFIAP-1, HFIAP-2, HFIAP-3) were purified and characterized. Using tandem electrospray ionization mass spectrometry, an unknown residue was identified as BrTrp. Chemical synthesis of sequences containing unmodified tryptophan was achieved using Fmoc chemistry and the peptides were observed to have antimicrobial activity against Gram-positive and Gram-negative aerobic and anaerobic bacteria. This family of peptides was designated as cathelicidins.

The cathelicidins originally discovered in mammals, are a gene family characterized by a remarkably conserved precursor region and a highly variable antimicrobial peptide domain. They include a group of cationic and usually amphipathic peptides that display a variety of activities related to host defense functions, among which the most acknowledged is a direct antimicrobial activity against various microbial pathogens (Tomasinsig and Zanetti, 2005). All members of this family are synthesized as precursors characterized by an N-terminal cathelin-like domain, which is relatively well conserved in evolution-

ary distant vertebrates as well. By contrast, the C-terminal region, which carries the active peptide, appears to be a focus for genetic mechanisms that have selectively generated considerable sequence diversity.

Later studies (Shinnar et al., 2003) showed that these peptides are produced as inactive precursors. Signal peptidase removes the N-terminal signal sequence, while peptidylglycine alpha-amidating monooxygenase often amidates and cleaves the C-terminal region. Removal of the cathelin domain liberates the active antimicrobial peptide. For mammalian sequences, this cleavage usually occurs through the action of elastase, but other tissue-specific processing enzymes may also operate. Once released, these bioactive peptides are susceptible to proteolytic degradation. It was proposed that some mature cathelicidins are naturally resistant to proteases due to their unusual primary structures. Some cathelicidins feature a high proportion of certain amino acids in their encoded antimicrobial peptides. The HFIAP family is distinguished by the presence of BrTrp produced by post-translational modification (Shinnar, 2003a). BrTrp does not appear to affect the antimicrobial activity or mechanism of interaction with lipid membranes exhibited by hagfish cathelicidins. Based on molecular modeling studies with chymotrypsin, BrTrp would not bind as well as native tryptophan in the active site of serine proteases, due to steric factors. The amino acid BrTrp may render the active peptides less susceptible to proteolysis for steric reasons. These suggest that the unusual primary structures of cathelicidin antimicrobial peptides may render them less susceptible to endogenous proteases, thereby extending their pharmacokinetic lifetimes and sustaining their bioactivity in vivo.

5.2. Styelins

Styelin D, a 32-residue, C-terminally amidated antimicrobial peptide, was isolated from the blood cells (hemocytes) of the solitary ascidian *Styela clava*. The peptide exhibited activity against Gram-negative and Gram-positive bacteria and was hemolytic and quite cytotoxic to eukaryotic cells (Taylor et al., 2000). Styelin D was remarkable in containing 12 post-translationally modified residues, including a 6-BrTrp, two monohydroxylysines, four 3,4-dihydroxyphenylalanines (DOPA), four dihydroxylysines and one dihydroxyarginine. The primary sequence of one variant was found to be: GW*LR**K**AAK**SVGK**FY*Y*K**HK*Y*Y*IK* AAWQIGKHAL-NH2, where W* is 6-BrTrp, R** is dihydroxyarginine, Y* is 3,4-dihydroxyphenylalanine, K* is 5-hydroxylysine, and K** is dihydroxylysine.

Tunicates rely on innate immunity and their hemocytes are important contributors to the host defense. *Styela clava*, a solitary ascidian, has eight hemocyte subtypes. Extracts of their total hemocyte population contained multiple small (2–4 kDa) antimicrobial peptides (Lehrer et al., 2003), one of which is the styelins. They are phenylalanine-rich, 32 residue peptides with activity against marine bacteria and human pathogens. Styelin D was one of the five styelins identified by peptide isolation and cDNA cloning.

A polypeptide containing the post-translationally modified amino acid L-6-BrTrp was isolated from the morula cells of the vanadium-accumulating ascidian, Phallusia mammillata (Taylor et al., 1997). The polypeptide, designated Morulin Pm, has a mol. wt. of 38255 and a simple amino acid composition consisting mainly of TOPA and 6-BrTrp, as well as Ser, Leu, Phe, and Ala. This is the first reported example of multiple sites of brominated tryptophans in a polypeptide of this size. Edman degrdation revealed the N-terminal sequence to be L-6-BrTrp-Leu-Phe-L-6-BrTrp before sequencing was blocked.

5.3. Conotoxins

Conotoxins are obtained from the toxin sacs of predatory snails of the species *Conus* found mainly in warm Pacific waters around Australia. The snails use their venom to immobilize and kill fish, shellfish, and marine worms. The toxic venom contains up to 50 different peptides that selectively inhibit the function of ion channels involved in the transmission of nerve signals in animals. Each of the 700 *Conus* species contains a unique set of peptides in their venom. These are small highly constrained and specialized polypeptides that can be synthesized chemically in quantities sufficient for research use. They are short peptides of 15–40 amino acids held in very tight conformations by multiple disulfide bridges. These patterns of disulfide bridges help to define a number of structural classes of conotoxins. The disulfide bridges are responsible for the highly constrained conformation of these toxins enabling the molecules to block a variety of different receptors with high binding affinity and selectivity. The conotoxins also have high levels of other modified amino acids including hydroxyproline, γ-carboxyglutamic acid and L-6-BrTrp. The first report of tryptophan residues in peptides/proteins being modified in a eukaryotic system and of halogenation of tryptophan in vivo was published in 1997 (Craig et al., 1997). It described a post-translational modification involving bromination of tryptophan in peptides recovered from the venom of carnivorous ma-

Bromocontryphan
(Trp* = L-6-BrTrp)

Fig. 6. The unusual structure of the octapeptide bromocontryphan

rine cone snails. It was observed that a number of peptides gave no phenylthiohydantoin amino acid derivatives during chemical sequence analysis, and the observed mass was approximately 265 Da above that predicted. In addition, these peptides had intense absorption in the UV region at 280 nm and an unusual intact molecule mass isotopomer distribution. The residue, L-6-BrTrp, was identified in the sequence of the heptapeptide: Pca-Cys-Gly-Gln-Ala-Trp*-Cys-NH_2 (Trp* = L-6-BrTrp) isolated from *Conus imperialis*, a worm-hunting cone. The sequence of the peptide was determined using a combination of mass spectrometry, amino acid, and chemical sequence analyses. The precise structure and stereochemistry of the modified residue was determined as L-6-BrTrp by synthesis, co-elution, and enzymic hydrolysis experiments (Craig et al., 1999).

The same researchers demonstrated unequivocally that post-translational bromination of a tryptophan residue occurs in the biological active octapeptide bromocontryphan, purified and characterized from *Conus radiatus* venom (Jimenez et al., 1997) (Fig. 6). This unusual octapeptide has several post-translational modifications as follows (see asteriscs): proteolytic cleavage at the N-terminus, hydroxylation of Pro, epimerization of Trp, bromination of Trp, and C-terminal amidation.

Clones encoding bromocontryphan were identified from a cDNA library made from *C. radiatus* venom ducts. The mRNA sequence obtained predicts a prepropeptide, which has the mature peptide sequence at the C-terminal end, with the L-6-BrTrp residue encoded by UGG, the tryptophan codon. These data provide the first direct evidence for post-translational bromination of a polypeptide translated through the normal cellular machinery. The overall result is a molecule, which closely resembles marine natural products produced through specialized biosynthetic pathways, comprising many enzyme-catalyzed steps.

A 41-amino acid peptide, α-conotoxin GVIIIA, which can inactivate the 5-HT_3 receptor, an excitatory serotonin-gated ion channel, was also purified (England et al., 1997). α-Conotoxin contains also a brominated tryptophan residue, which may be important for peptide activity because the endogenous ligand for the 5-HT_3 receptor is a hydroxylated tryptophan derivative.

L-6-BrTrp was later purified and identified in a 33-amino acid peptide from the venom of the fish-hunting *Conus radiatus*. This peptide induces a sleep-like state in mice of all ages and is referred to as bromosleeper, ''light sleeper'' or the r7a conotoxin. Three residues of the post translationally modified amino acid, 6-BrTrp has been characterized in this peptide (Jimenez et al., 2004). The light sleeper peptide equilibrates slowly between two distinct conformers, and has four γ-carboxyglutamate residues. The pattern of post-translational bromination in the light sleeper peptide suggests that tryptophan residues at N- and C-termini may be preferential sites for post-translational bromination.

Other conopeptides having 6-45 amino acids, including one or more BrTrp residues were prepared (Cruz et al., 1998). These BrTrp containing conopeptides are useful as antihelminthic agents, anti-vomiting agents, sleep inducing agents, adjuncts to anesthesia, anticonvulsant or neuroprotective agents.

In 1999 the T-superfamily of conotoxins was discovered and eight different T-superfamily peptides from five *Conus* species were identified and partially characterized (Walker et al., 1999). T-Superfamily peptides are a potentially large and diverse group of biologically active peptides, widely distributed in the 500 different *Conus* species. Although the peptides are small (11–17 amino acids), their sequences are strikingly divergent, with different peptides exhibiting varying extents of post-translational modification. These peptides are among the smallest of the multiply disulfide-bonded conotoxins, with four of the amino acids being highly conserved Cys residues. The peptides that have been extensively characterized are as follows: p5a, (GCCP-KQMRCCTL*); tx5a, (γCCγDGW + CCTAAO); and au5a, (FC-CPFIRYCCW) where γ = γ-carboxyglutamate, W+ = 6-BrTrp, O = hydroxyproline, T = glycosylated threonine, and * = COOH-terminal amidation.

Two highly modified conotoxins from the mollusk *Conus textile*, ε-TxIX and Gla(1)-TxVI (Scheme 7), were later characterized by matrix-assisted laser desorption/ionization (MALDI) and electrospray mass spectrometry. Characterization was also achieved by electrospray ionization tandem and triple mass spectrometry in combination with enzymic cleavage and chemical modification reactions (Kalume et al., 2000). The mass spectrometric studies allowed the confirmation of the sequence determination by Edman degradation and the assignment of unidentified amino acid residues, among which 6-BrTrp residues and an O-glycosylated threonine residue were observed. MS/MS analysis indicated that Gla(1)-TxVI includes two BrTrp residues, one in position 3 and the

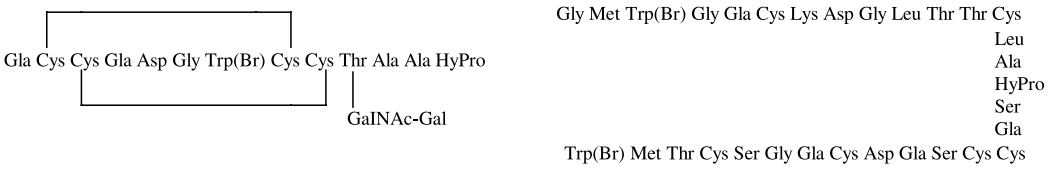


Fig. 7. Primary structures of conotoxins ε-TxIX (left) and Gla(1)-TxVI (right)

other at the C-terminus (position 31). It was claimed to be the first known conotoxin having two BrTrp residues.

At about the same time, another peptide was purified from *Conus textile* venom, which caused hyperactivity in mice (Lirazan et al., 1999). The 31-amino acid peptide has six residues with unusual post-translational modifications: four γ-carboxyglutamates and two brominated tryptophan residues. It was the first known gene product with multiple bromotryptophan residues.

Still another peptide, de13a from the crude venom of *Conus delessertii* collected in the Yucatan Channel, Mexico, was isolated and purified. The peptide has a high content of posttranslationally modified amino acids, including 6-BrTrp (Aguilar et al., 2005). The sequence analysis together with cDNA cloning and a mass determination (monoisotopic mass of 3486.76 Da), established that the toxin has the sequence DCOTSCOTTCANGW* ECCKGYOCVNKACSGCTH*, where W* is 6-BrTrp.

A recent report describes the chemical synthesis and NMR characterization of the 13-amino acid glycopeptide tx5a (Gla-Cys-Cys-Gla-Asp-Gly-Trp*-Cys-Cys-Thr*-Ala-Ala-Hyp-OH), where Trp* = 6-BrTrp and Thr* = Gal-GalNAc-threonine, which were isolated from *Conus textile* (Kang et al., 2004). This peptide has the greatest diversity of post-translational modifications found in any conotoxin hitherto. There are two disulfide crosslinks, a hydroxylated proline residue, a brominated tryptophan residue, and two γ-carboxylated glutamic acid residues. The glycopeptide tx5a also contains a disaccharide composed of N-acetylgalactosamine (GalNAc) and galactose (Gal).

5.4. 5-BrTrp and Sickle-Hb polymerization

Sickle cell disease is a group of inherited red blood cell disorders. Sickle red blood cells become hard, sticky and shaped like sickles used to cut wheat. When these hard and pointed red cells stream through the blood vascular, they clog the flow and break apart. This can cause pain, damage and a low blood count, or anemia. Polymerization of deoxy sickle cell hemoglobin S (Hb S) within the erythrocytes is the primary molecular event linked to sickle cell disease.

The effects of 42 β-aryl-substituted alanines on the inhibition of gelation of sickle Hb S were evaluated (Poillon, 1982). It was found that bicyclic aromatic nuclei are considerably more potent than monocyclic ones and the nature of the substituent at a fixed position on a particular aromatic ring exerts a profound effect on the expression of anti-gelling activity. 5-BrTrp was found to be the best gelation inhibitor, with 5 times more effectiveness than phenylalanine. It was proved later that tryptophan and 5-BrTrp are potent inhibitors of sickle-Hb polymerization. Dipeptides containing 5-BrTrp were also prepared and evaluated for anti-gelation activity of Hb S from sickle cell anemia patients (De Croos et al., 1990). H-(5-BrTrp)$_2$-OH was found to be the most potent, with 5.9 times the activity of tryptophan. Other less active peptides were H-5-BrTrp-Trp-OH and H-Trp-5-BrTrp-OH.

The binding sites of indole-based gelation inhibitors with sickle cell hemoglobin were later investigated by two parallel theoretical approaches (Manavalan et al., 1992). A geometric approach originated by Kuntz and co-workers uses a spatial buildup scheme to locate potential binding regions, while a hybrid grid/geometric search method, searches for specific indole ring binding pockets over the hemoglobin surface. The binding sites derived from these calculations were tested for their ability to accommodate indole rings by means of accessibility calculations with probes of various radii. These sites were further scanned for van der Waals overlap and electrostatic interactions. A full 5-BrTrp residue was built in each indole ring binding site, and its conformational energy of association with sickle cell hemoglobin was calculated at that site. These theoretical results predict a total of 14 potential binding regions, including all of the sites observed from X-ray crystallography and sites that are consistent with solution nuclear magnetic resonance studies.

Another investigation of the indole-based binding sites utilized the technique of photoaffinity labeling (Li et al., 1995). The cyanomet forms of HBA and HBS were subjected to photoaffinity labeling with Nα-(4-azidotetrafluorobenzoyl) tryptophan and Nα-(1-ethyl-2-diazomalonyl)-5-BrTrp respectively. Both irradiated samples of HBA and HBS were denatured, digested with trypsin, and then

separated by reversed-phase HPLC. A labeled tryptic peptide was isolated from the photolabelling of HBS with Nα-(1-ethyl-2-diazomalonyl)-5-BrTrp. The peptide was identified to be Val1(α)-Lys7(α), with the label attached to Val1(α), by virtue of amino acid analysis and sequencing, in conjunction with fast-atom-bombardment MS. The binding mode of Nα-(1-ethyl-2-diazomalonyl)-5-BrTrp and its relevance to the potency of the 5-BrTrp-based anti-sickling agents was proposed.

It was later shown that deoxy sickle Hb S exhibits binding sites for a limited number of small molecules (Prabhakaran et al., 2001). A modeling study was performed to examine binding of the following molecules to Hb: *DL*-5-BrTrp tripeptides, H-(5-BrTrp)-NH_2, H-(5-BrTrp)$_2$-NH_2, H-(5-BrTrp)-Gly-BrTrp-NH_2 and H-(5-BrTrp)-Aib-5-BrTrp-NH_2, with increased flexibility as well as rigidity. In addition, the oxygen affinity and polymerization of Hb S in the presence of these peptides were measured. Results showed that these peptides had a very limited influence on the oxygen affinity of Hb S, but exhibited very strong inhibitory effects on the polymerization. In the presence of 2 equivalents of 5-BrTrp-Gly-5-

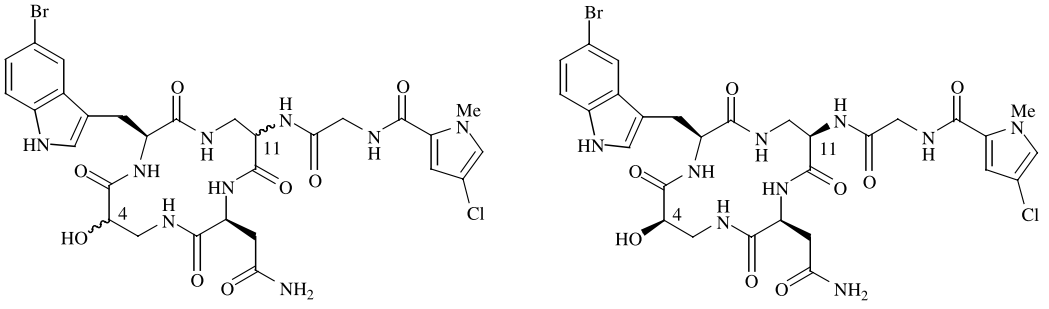
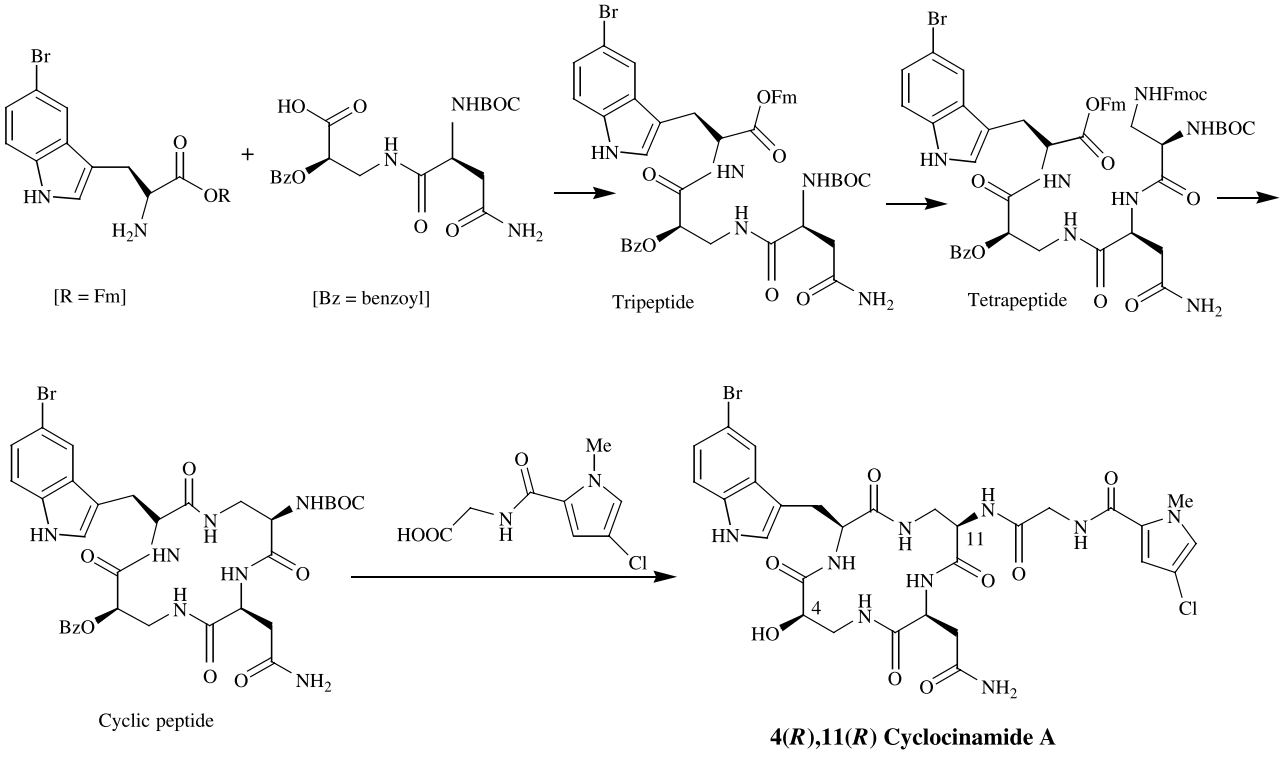


Fig. 8. Cyclocinamide A and its diasteroisomer



Scheme 29. Synthesis of 4(*R*),11(*R*)-cyclocinamide A (Grieco and Reilly, 1998)

BrTrp-NH_2, the polymerization of deoxy Hb S is nearly twice that of a control sample of deoxy Hb S. This is the best inhibitory effect seen so far for any noncovalent inhibitor of the polymerization.

5.5. Other peptides

Cyclocinamide A is a cytotoxic hexapeptide isolated from the methanolic extracts of the marine sponge *Psammocinia* sps. (Thorectidae). The synthesis of 4(*R*),11(*R*)-cyclocinamide A was performed with the purpose of determining the absolute stereochemistry at C(4) and C(11) (Grieco and Reilly, 1998) (Fig. 8).

The first step in this total synthesis includes the coupling of fluorenylmethyl ester of (*S*)-5-BrTrp with N_α-BOC-(*S*)-Asn-O-benzoyl-(*R*)-isoserine, yielding a tripeptide. Cleavage of the BOC group followed by coupling with N_α-BOC-N_β-Fmoc-(*R*)-2,3-diaminopropanoic acid, afforded a tetrapeptide, which is transformed into a cyclic peptide. Cleavage of the BOC is followed by coupling with the glycine derived side chain and removal of the benzoyl group to yield the 4(*R*),11(*R*)-cyclocinamide A. The later is not identical to the natural analogue, suggesting that the latter possesses 4(*S*), 7(*S*), 11(*S*), 14(*S*) configuration (Scheme 29).

The highly potent corticotrophin analogs [5-bromotryptophan^9]-β-coritcotrophin-(1-24)-tetracosapeptide, was prepared by using standard methods of stepwise and fragment condensation (Allen et al., 1980). In an isolated adrenal cell bioassay, the peptide had steroidogenic potency 2.4 times that of Synacthe.

6. Conclusions

The bromination of tryptophan and its derivatives appears to occur widely in marine organisms, especially in sponges, tunicates and algae. These brominated compounds arise through a broad spectrum of post-translational modification enzymes, e.g. bromo- and lactoperoxidases. In a large variety of these natural products, the bromamino acid is part of the peptidic framework. Some are pure peptides, composed of common amino acids. Others are cyclic peptides, depsipeptides, or some kind of hybrid (e.g. polyketide/polypeptide). These natural products are likely to be defensive compounds, endowed with strong pharmacological activity. Although a large volume of research has already been done in this field, we are only starting to reveal the huge library of pharmacologically active BrTrp-containing compounds. Many questions are still to be answered and an advanced research is very much needed. We will mention only few important issues:

a) Intensive research is still needed on isolation and characterization of BrTrp-containing peptidic and non-peptidic compounds.
b) The various biosynthetic routes leading to the in vivo formation of BrTrp derivatives are still vaguely understood.
c) The question of why marine organisms recruit bromination as a mean of increasing their peptidic repertoire, is not yet answered.
d) Mode of toxic activity of the 6-BrTrp peptides as a venom must be elucidated.
e) What is the role and effects of the bromine atom? steric? electronic? configuration wise?
f) A comprehensive study is still needed on the biological activity, inhibitory influence and medicinal feasibility of the various BrTrps.
g) The development of sophisticated analytical and spectroscopic methods for the detection and quantization of the different BrTrps and derivatives is intensely needed.

We trust that this review will provide the input to interest new and old comers to this fascinating subject, and to motivate intensive research on the chemistry and biology of these exciting compounds.

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