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Redox *N*-Alkylation of Tosyl Protected Amino Acid and Peptide Esters†,‡

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Condensation of N^{α}-tosylated amino acid and peptide esters with alcohols (MeOH, EtOH, ^{i}PrOH) in the presence of the triphenylphosphine–diethyl azodicarboxylate adduct produced excellent yields of the corresponding N^{α}-alkylated derivatives. Optically pure N^{α}-alkylamino acids can only be obtained from methyl and benzyl esters using iodotrimethylsilane and hydrogenolysis, respectively, for the carboxy-deprotection and sodium in liquid ammonia for the amino-deprotection. That carboxy-deprotection of methyl esters by saponification is accompanied by racemization was established by high-performance liquid chromatography studies. Alkylation rates and yields for the reactions examined were found to depend only on the relative positions of the tosylamino and the carboxy functions. Removal of the carboxy group from the α-position resulted in longer reaction times and significant decreases in the yield of the desired *N*-alkylated derivatives. Accordingly, tosyl-protected lysyl and ornithyl side-chains of fully protected amino acids and peptides were selectively *N*-methylated in moderate yields in the presence of other amino functions bearing the *tert*-butoxycarbonyl (Boc) group which is commonly used for protection in peptide synthesis.

In connection with our studies aimed at the preparation of chiral *N*-tosylamino alcohols from naturally occurring amino acids (AAs), through $LiAlH_4$ reduction of the corresponding N^{α}-tosylamino acid methyl esters (TAAMs), we decided to synthesize the latter compounds employing the Mitsunobu reaction.[2] Preliminary experiments were carried out with *N*-tosylalanine (Tos-Ala, **1**), readily available via *N*-tosylation[3] of Ala, which was subjected to Mitsunobu-type esterification using an excess of the system MeOH–triphenylphosphine (TPP)–diethyl azodicarboxylate (DEAD). Thin layer chromatographic (TLC) examination of the reaction mixture revealed that in addition to the expected ester **2**, a non-polar by-product was simultaneously formed. This was finally identified as the corresponding *N,O*-dimethyl derivative **3**. This unexpectedly facile formation of **3** prompted us to investigate the applicability of Mitsunobu reaction to the synthesis of *N*-alkylated amino acids (AAAs) and derivatives.

N^{α}-Methylamino acids (MAAs) are naturally occurring compounds frequently encountered either in the free state,[4] or bound within biologically important peptides,[5] such as the immunosuppressive cyclic undecapeptide cyclosporine, and some cyclodepsipeptides of marine origin, such as the didemnins[6] and the dolastatins,[7] with significant antibiotic and antineoplastic activity. In addition, MAAs have been shown to be valuable tools in structure–activity relationship studies of biologically important peptides such as angiotensin,[8] enkephalin,[9] bradykinin[10] and luteinizing hormone-releasing hormone,[11] and the pyrimidine nucleoside peptide antibiotic polyoxins[12] and the mercaptopeptide inhibitors of collagenases.[13] Thus, incorporation of MAAs in such compounds has led to analogues with enhanced potency and duration of action, attributed to their reduced hydrolytic lability to proteases, strong antagonistic activity in some cases, and altered affinity for target enzymes compared with the parent compounds. Owing to their significance, a variety of synthetic methods have been developed which

* All amino acids in this work are of the *S*-configuration unless otherwise stated.

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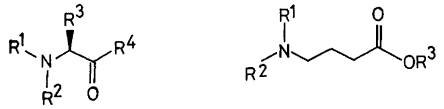
allow the preparation of homochiral MAAs and derivatives suitable for use in peptide synthesis.[14] These methods usually involve application of strong bases (e.g., NaOH, NaH) to chiral *N*-protected AAs with the tosyl or urethane-type protecting groups, e.g., *tert*-butoxycarbonyl (Boc) and benzyloxycarbonyl (Cbz), followed by MeI treatment, or two consecutive reductive alkylation steps of amino acids with benzaldehyde and formaldehyde. These types of alkylation reaction are accompanied by various degrees of racemization, optically pure products being obtained only by using the Tos or Boc/Cbz group for *N*-protection and NaOH or NaH respectively as the base.[14] Other, less general, methods have been reported including methylation of diphenylphosphinamides of amino acids,[15] enzymatic resolution of suitable racemic MAA derivatives,[16] asymmetric synthesis using chiral templates incorporating *N*-methylamino functions[17,18] and free-radical-mediated *N*-methylation of carbamate derivatives of AAs.[19]

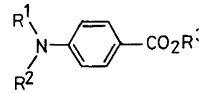
Results and discussion

The present paper reports on the N^{α}-alkylation of AA and peptide derivatives, under neutral and mild reaction conditions, and the extension of the thus developed protocol to accommodate selective N^{ω}-methylation of peptides incorporating the diamino acids lysine (Lys) or ornithine (Orn). The key step in the present methodology is the condensation of TAA and peptide esters with alcohols in the presence of the redox condensation system TPP–DEAD. Structures of the compounds encountered in the work on N^{α}-alkylation and N^{ω}-alkylation are to be found in Figs. 1 and 2, respectively.

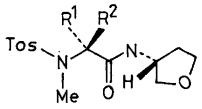


	R^1	R^2	R^3	R^4
1	Tos	H	Me	OH
2	Tos	H	Me	OMe
3	Tos	Me	Me	OMe
4	Tos	H	^{S}Bu	OH
5	Tos	H	^{S}Bu	OMe
6	Tos	Me	^{S}Bu	OMe
7	Tos	H	^{t}Bu	OMe
8	Tos	H	^{i}Pr	OMe
9	Tos	Me	^{t}Bu	OMe
10	Tos	Me	^{i}Pr	OMe
22	Tos	Et	Me	OMe
23	Tos	Et	^{S}Bu	OMe
24	Tos	Et	^{t}Bu	OMe
25	Tos	^{i}Pr	Me	OMe
26	Tos	^{i}Pr	^{S}Bu	OMe
29	Tos	H	^{S}Bu	OBzl
30	Tos	H	^{i}Pr	OBzl
31	Tos	Me	^{S}Bu	OBzl
32a	Tos	Me	^{i}Pr	OBzl
33	Tos	^{i}Pr	^{i}Pr	OBzl
34	Tos	H	Me	Leu-OMe
35	Tos	H	Me	Phe-Leu-OMe
36	Tos	Et	Me	Leu-OMe
37	Tos	Me	Me	Phe-Leu-OMe
38	H	Me	^{S}Bu	OH
39	H	^{i}Pr	^{i}Pr	OH
40	H	Me	Me	NH_2
41	H	Et	Me	Leu-NH_2
42	H	Me	Me	Phe-Leu-OH
43	Boc	H	Me	OH
44	Cbz	H	Me	OH
45	Boc	H	Me	OMe
46	Cbz	H	Me	OMe
47	Boc	Me	Me	OMe
48	Cbz	Me	Me	OMe

	R^1	R^2	R^3
16	Tos	H	H
18	Tos	H	Me
20	Tos	Me	Me



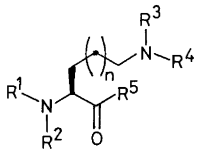
	R^1	R^2	R^3
17	Tos	H	H
19	Tos	H	Me
21	Tos	Me	Me



	R^1	R^2
27	H	^{i}Pr
28	^{i}Pr	H

Fig. 1. Structures of compounds encountered in the N^{α}-alkylation of N^{α}-tosylamino acid and peptide derivatives.

N$^{\alpha}$-*Alkylation.* Thus, treatment of Tos-Ala (**1**) or Tos-Ile (**4**), available by means of *N*-tosylation of alanine (Ala) or isoleucine (Ile), with 10 equivalents (equiv.) of MeOH in the presence of 1.2 equiv. each of TPP (triphenylphosphine) and DEAD (diethyl azodicarboxylate) in THF resulted in the production of a mixture consisting of unchanged starting material and two products, namely the esters **2** or **5** and the dimethylated derivatives **3** or **6**, respectively. Addition of a further 1.2 equiv. each of TPP and DEAD, and careful examination of the reaction mixture by TLC showed complete, very fast, reaction of starting material to products, followed by quantitative conversion, slower compared with esterification, of esters **2** or **5** into the *N*-methylated derivatives **3** or **6**, respectively. These experiments indicated that esterification and N^{α}-alkylation take place at comparable rates and therefore chemospecific methylation, at the carboxy group, cannot be performed under these reaction conditions. This was taken to mean that N^{α}-alkylation of the tosylamino function, with alcohols other than MeOH, should be performed on the preformed methyl ester. Thus difficult saponifications involving enhanced danger of racemization, in order to deprotect the carboxy function, would be avoided. On the other hand, when these experiments were repeated using 2.5 equiv. each of TPP and DEAD from the beginning of the reaction, complete conversion of starting material into the dialkylated product was realized within 1 h at room temperature and the expected products **3** and **6** were obtained in 96 and 87% yields, respectively, after purification by routine flash column chromatography (FCC).



	n	R^1	R^2	R^3	R^4	R^5
49	2	Boc	H	Tos	H	OH
50	2	Boc	H	Tos	H	OMe
51	2	Boc	H	Tos	Me	OMe
52	2	Tos	H	Boc	H	OH
53	2	Tos	Me	Boc	H	OMe
54	2	Boc	H	Tos	H	Gly-Gly-Phe-Leu-OMe
55	2	Boc	H	Tos	Me	Gly-Gly-Phe-Leu-OMe
56	2	Boc	H	Tos	Me	OH
57	1	Boc	H	Tos	H	OH
58	1	Boc	H	Tos	Me	OMe
59	1	Boc	H	Tos	H	OMe
60	1	Boc	H	Tos	H	Ala-OMe
61	1	Boc	H	Tos	Me	Ala-OMe

Fig. 2. Structures of compounds encountered in the N^{ω}-methylation of N^{ω}-tosyldiamino acid and peptide derivatives.

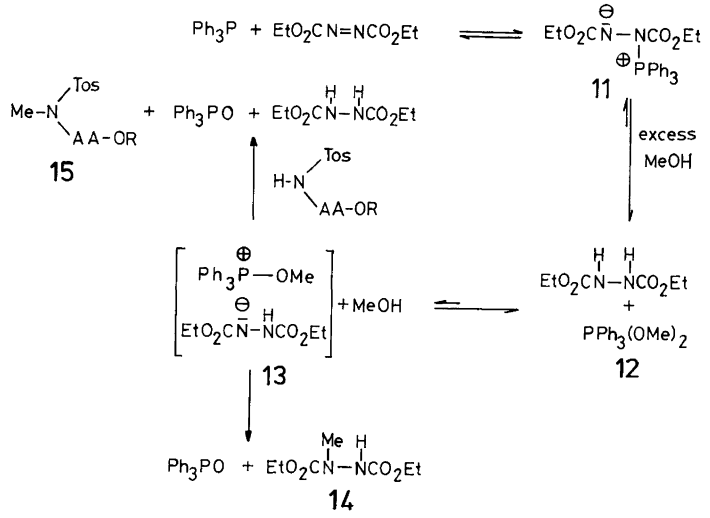


Fig. 3. Mitsunobu-type *N*-methylation of tosylamino esters with excess methanol.

Following these observations, the *N*-alkylation of TAAMs, such as Tos-Ala-OMe (**2**), Tos-Ile-OMe (**5**), Tos-Leu-OMe (**7**) and Tos-Val-OMe (**8**) with a variety of alcohols (e.g., MeOH, EtOH, ^{i}PrOH) was examined. These esters were readily available through esterification[20] of the parent AAs, Ala, Ile, leucine (Leu) and valine (Val), followed by *N*-tosylation with TosCl–Et_3N in $CHCl_3$ at 0 °C. Initial experiments with MeOH, which led to the production of the corresponding *N*-methylated derivatives **3**, **6**, **9** and **10**, respectively, showed that the presence of a large excess (tenfold) of MeOH not only does not facilitate the reaction but, on the contrary, results in retarded reaction rates and incomplete reaction. It is thus plausible to suggest that the large excess of MeOH transforms the initially formed TPP–DEAD adduct (**11**, Fig. 3), into the intermediate dimethoxytriphenylphosphorane (**12**) which, in turn, slowly collapses to MeOH and the methoxytriphenylphosphonium salt **13**.[21,22] In the absence of a sufficiently strong acid component, that is with a pK_a lower than 11, *N*-methylation of half-protonated DEAD (DEADH) to give **14** effectively competes with the formation of the desired *N*-methylated product **15**.

When, however, 1.5 equiv. each of MeOH, TPP and DEAD were initially used, followed by an additional 0.5 equiv. after 5 min at room temperature, excellent yields of *N*-methylated products **3**, **6**, **9** and **10** were realized. Taking into consideration that *N*-alkylsulfonamides such as TosNHMe (pK_a 11.7) can be alkylated with alcohols, albeit in moderate yields,[23,24] the facile and high-yielding *N*-methylation of the TAAMs can be attributed to the presence of the electron-withdrawing carboxy group in the α-position to the tosylamino function, which serves to lower its pK_a value. To verify this hypothesis, the *N*-tosylated derivatives **16** and **17** of γ-aminobutyric acid (GABA) and *p*-aminobenzoic acid (PABA) were prepared and treated with 1.5 equiv., followed by another 1.0 equiv. after 1 h at room temperature, each of MeOH, TPP and DEAD. TLC examination of the reaction mixtures showed that although ester formation was equally fast for both substrates, the intermediate esters produced, namely **18** and **19**, reacted differently with the excess reagents present. Thus, *N*-methylation took place along with esterification from the beginning of the reaction period, but completion of *N*-methylation, after completion of esterification, was observed only for the PABA case resulting in an 85% yield of **21**. *N*-Methylation of the GABA derivative **18** was slow and required additional portions of reagents to provide a good yield (78%) of **20** and unchanged ester **18** (20% recovery). It is thus evident that removal of the carboxy group from the α-position of the tosylamino function leads to slower *N*-methylation and incomplete reaction. The same trend is also followed in the N^{ω}-alkylation experiments described below. In the case of PABA, the mesomeric effect is transmitted through the aromatic ring and at least preserves the required acidity of the tosylamino function. It should be noted here that the *N*-tosylamino function can be made sufficiently acidic for participation in high-yielding Mitsunobu alkylation reactions by attaching another electron attracting group, e.g., of the carbamate type,[25] to the nitrogen atom.

Having established the conditions required to effect efficient N^{α}-methylation, we turned our attention to the condensation of TAAMs with other alcohols. Thus, reaction of esters **2**, **5** and **7** with EtOH and ^{i}PrOH, under conditions identical with those used to *N*-methylate these esters, led to the preparation of the expected N^{α}-ethyl derivatives **22**, **23** and **24** and the N^{α}-isopropyl derivatives **25** and **26**, respectively, in high yields. N^{α}-Ethylamino acids (EAAs) have been prepared from *N*-acetylamino acids by treatment with trimethyloxonium tetrafluoroborate followed by $NaBH_4$ reduction of the thus obtained imino ethers, or reductive alkylation under hydrogenation conditions.[26] Both methods, however, involve danger of racemization, whereas direct ethylation of *N*-protected

AAs is far from satisfactory due to a β-elimination reaction of the ethylating agent.[26] Of course direct *N*-isopropylation with isopropylating agents can be expected to be even less favourable. During the course of the present investigation the alkylation, in rather low yields, of azaglycine-containing peptides with a variety of alcohols under Mitsunobu-type reaction conditions was reported.[27]

The applicability of the Bzl group for carboxy protection was examined for the condensation of the *N*-tosylamino acid benzyl esters (TAAB) **29** and **30** with MeOH or ^{i}PrOH, and unexceptionally high yields of the N^{α}-alkylated derivatives **31–33** were found. We wish to draw special attention to the high-yielding synthesis of derivatives **26** and **33** which clearly indicates that steric congestion is not at all an important factor in the present alkylation reaction, which can indeed accommodate bulky alcohols even in the presence of extreme steric hindrance from the AA side chain. These results, in combination with the fact that the well established *N*-detosylation with Na in liquid ammonia is free of racemization,[28] show that the present protocol constitutes a general and efficient method of preparing alkyl amino acids (AAAs), where the alkyl group can be varied at will, under extremely mild reaction conditions. Furthermore, this methodology can be successfully applied to alkylate peptides tosylated at the *N*-terminus (TPMs) as is shown by the efficient *N*-alkylation of the dipeptide and tripeptide derivatives **34** and **35**, respectively. The expected *N*-alkylated peptide derivatives **36** and **37** were thus obtained in 85 and 78% yields. The required substrates **34** and **35** were readily obtained by routinely coupling **1** with H-Leu-OMe and H-Phe-Leu-OMe hydrochlorides, respectively, in the presence of *N*-methylmorpholine (NMM) and isobutyl chloroformate (mixed anhydrides coupling method).

Racemization studies. Because we were aware that saponification of fully protected MAAs can be accompanied by racemization,[29] we decided to examine the configurational fate of derivatives such as **10** on saponification. Thus, **10** was treated with aqueous NaOH in methanol at room temperature and the resulting acid was coupled with (*R*)-3-aminotetrahydrofuranyl tosylate (*R*-ATHF),[30] using benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP)[31] as the condensing agent, to provide amide **27** the diastereomeric purity of which was questionable. Amide **27** was also prepared by carboxy-deprotection of ester **10** with iodotrimethylsilane (TMSI).[32] For reference purposes, diastereomerically pure amides **27** and **28** were obtained from (*S*)- and (*R*)-Val, respectively, as follows. These AAs were routinely esterified with benzyl alcohol[33] and the resulting benzyl (Bzl) esters *N*-tosylated with TosCl–Et_3N in $CHCl_3$ at 0°C. These *N*-tosylated derivatives were subsequently *N*-methylated with MeOH–TPP–DEAD, to provide esters **32a** and **32b**, respectively, and then carboxy-deprotected by catalytic hydrogenolysis in glacial AcOH, at room temperature in the presence of 10% Pd on activated C. Finally, coupling of the free enantiomeric acids with (*R*)-ATHF provided the diastereomeric amides **27** (*S*,*R*) and **28** (*R*,*R*), respectively. High-performance liquid chromatography (HPLC) showed (Fig. 4) that the sample derived from (*S*)-Tos(Me)-Val-OBzl through carboxy-deprotection by hydrogenolysis and coupling with (*R*)-ATHF was indeed diastereomerically pure, as indeed was the sample derived from (*S*)-Tos(Me)-Val-OMe through carboxy-deprotection by TMSI splitting followed by coupling with (*R*)-ATHF. However, the sample derived from (*R*)-Tos(Me)-Val-OBzl, initially thought to be diastereomerically pure (e.g., **28**), was shown to contain as much as 12% **27**. Since the commercially available (*R*)-Val contained only about 0.2% (*S*)-Val and hydrogenolysis does not result in any racemization, formation of **27** must have occurred during coupling, which is a much slower reaction in this case. Finally, the sample derived from (*S*)-Tos(Me)-Val-OMe through carboxy-deprotection by saponification contained as much as 44% **28**, and thus considerable racemization took place during saponification. These studies showed that optically pure *N*-tosylated methylamino, and in general alkylamino, acids can only be obtained by the use of the Bzl

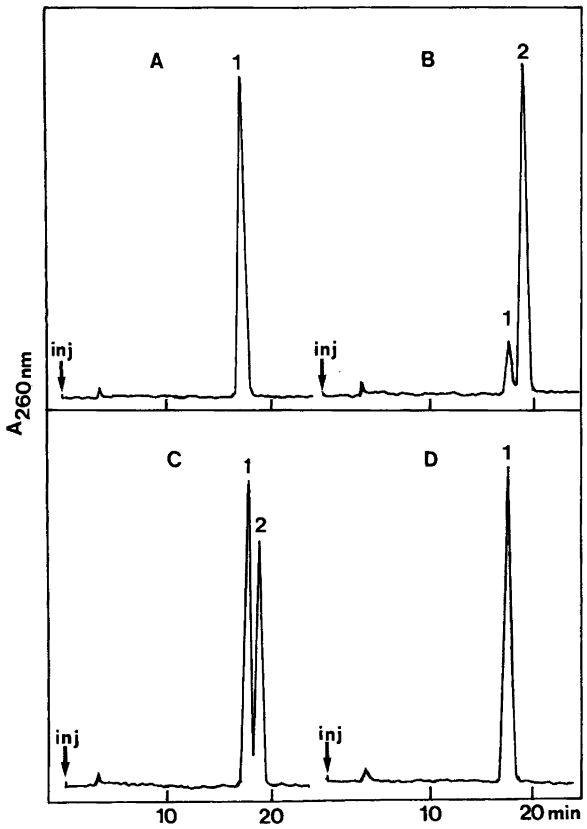


Fig. 4. HPLC of samples of (Tos,Me)-Val-ATHF of different origins: (A) hydrogenolysis of **32a**, (B) hydrogenolysis of **32b**, (C) saponification of **10**, (D) TMSI splitting of **10**. Column: 250×4.6 mm ID, packed with Lichrosorb SI-100, 5 μm particle size. Solvent: hexane–AcOEt–MeOH (40:6:1), flow rate 1.2 ml min^{-1}. Detection at 260 nm.

group for carboxy protection. Alternatively, the Me group can be used for carboxy protection but deprotection must then be performed at a later stage using TMSI.

N$^{\omega}$-*Alkylation of amino acids and peptides.* Having established the viability of N^{α}-alkylation of AA and peptide derivatives, and bearing in mind the problems associated with removal of the carboxy group from the tosylamino function, we turned our attention to side-chain alkylation of diamino acids such as Lys and Orn, and especially to peptides incorporating these amino acids. It must be noted that (*S*)-N^{ω}-methyllysine is a biologically important, naturally occurring compound found either free or bound within specialized proteins.[34] A variety of methods have been developed which provide access to N^{ω}-Me- and -Et-Lys and -Orn and derivatives[34] which are actually based on the methods used to synthesize AAAs. Mention should also be made of a quite recent method providing access to optically pure N^{ω}-Me-Lys and -Orn via N^{3}-protected (*S*)-3-aminolactams.[35] Prior to the examination of the applicability of the present method of alkylating tosylaminoalkyl side chains in peptides, it was considered of prime importance to establish its compatibility with α-amino functions protected by the commonly used protecting groups of the urethane type, e.g., Boc and Cbz. Thus, Boc-Ala (**43**) and Cbz-Ala (**44**) were treated with MeOH under reaction conditions identical with those used to alkylate **1** and the reaction mixtures were followed by TLC. Although esterification rapidly produced, as expected, the corresponding esters **45** and **46**, respectively, no N^{α}-methylation product, e.g., **47**, was obtained from **45** even after several days at room temperature and upon addition of several portions of the MeOH–TPP–DEAD reagent mixture, whereas in the case of **46** a 10% yield of **48** and ca. 90% of recovered ester **46** were obtained. The assignment for the structures of **46** and **48** was carried out only by FAB-MS and IR measurements. It was thus evident that although the Boc group could be safely used for N^{α}-protection, Cbz can only be safely used with sufficiently fast alkylation reactions. Since we were aware of the slow alkylation of tosylamino functions distant from a carboxy function, the Boc group was exclusively used for N^{α}-protection.

Initial experiments on the N^{ω}-alkylation of Lys derivatives were carried out with **49** and MeOH. Esterification was extremely fast, whereas N^{ω}-methylation was initially fast but became very sluggish during the progress of the reaction and could not be made to go to completion even after repeated additions of reagents. Obviously, as the concentration of the initially formed ester **50** decreases, methylation of DEADH competes favourably. FCC of the thus obtained reaction mixture afforded the pure ester **50** and N^{ω}-methylated ester **51** in 38 and 58% yields, respectively. In order to check whether the only factor responsible for this behaviour was indeed the relative position of the tosylamino and carboxy groups, the isomeric Lys derivative **52** was prepared and reacted under conditions identical with those used for **49**. It was thus shown that this particular derivative, being of the α-tosylamino acid type, reacted similarly to, e.g., **1** or **4** to produce **53** in 90% yield, and thus the distance between the tosylamino and carboxy group does indeed appear to be the only important factor for the efficiency of such reactions. Slow and incomplete N^{ω}-alkylations were also observed with the Lys pentapeptide **54** which afforded the expected product **55** in 55% yield and unchanged starting material **54** with 40% recovery. For the sake of comparison, **55** was also prepared through coupling of **56**, readily obtained from **51** by saponification, with the tetrapeptide H-Gly-Gly-Phe-Leu-OMe in the presence of PyBOP and had identical physical characteristics with those of the sample obtained by N^{ω}-methylation of **54**. Similarly, application of the method to the Orn derivative **57** produced a 60% yield of O,N^{ω}-dimethylated product **58** and 36% yield of ester **59**. Similar results were obtained when the dipeptide **60**, incorporating N^{α}-Boc-Orn, was subjected to identical reaction conditions to give a 70% yield of the N^{ω}-methylated dipeptide **61**. Dipeptide **60** was readily obtained by coupling the appropriate carboxy and amino components using PyBOP, whereas the pentapeptide **54** was obtained by coupling **49** with the tetrapeptide ester H-Gly-Gly-Phe-Leu-OMe using the mixed anhydride method (^{i}BuOCOCl–NMM). It should be pointed out here that the separation of product/unchanged methyl ester from the by-products of the reaction, namely triphenylphospine oxide (TPPO) and fully reduced DEAD ($DEADH_2$) or alkylated DEADH, may occasionally be a problem. In such cases, saponification of the mixture of products prior to separation is recommended.

Finally, as examples of the completion of the present methodology the AAAs **38** and **39**, the MAA amide **40**, the *N*-ethylated dipeptide amide **41** and the tripeptide **42**, were readily obtained in good overall yields (see the Experimental) from the corresponding fully protected derivatives **31**, **33**, **3**, **36** and **37** through routine hydrogenolysis of the benzyl esters and ammonolysis or saponification (in the case of **37**) of the respective methyl esters, followed by detosylation with Na in liquid ammonia.[36]

Conclusions

The present methodology allows for the efficient N^{α}-monoalkylation of amino acid and peptide derivatives and the selective N^{ω}-monoalkylation of diamino acid residues in fully protected peptide chains under mild and neutral reaction conditions. The method is very attractive although the N^{ω}-alkylation takes place with moderate yields and requires careful separation of the N^{ω}-alkylated product from the unchanged starting material. Of course for complex, expensive peptides the starting material can be recycled. Further work to improve the efficiency of the N^{ω}-monoalkylation of diamino acid residues in peptide chains, with MeOH and other higher alcohols, is now in progress.

Experimental

General. Capillary melting points were taken on a Büchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl–Zeiss precision polarimeter. IR spectra were recorded for KBr pellets or neat samples (for oily compounds) on a Perkin-Elmer 16PC FT-IR spectrophotometer. ^{1}H NMR spectra were obtained at 200.13 MHz on a Bruker AC-200 instrument using $CDCl_3$ as the solvent for the fully protected AAAs and APs, and D_2O for the fully deprotected AAAs: references were the peaks at δ 7.29 ($CHCl_3$) and 4.70 (HDO), respectively. FAB spectra were recorded on a Fisons-VG ZAB 2f instrument, operated at 8 keV accelerating potential, with an M-SCAN ion gun operated at 10 μA and 9 keV Xenon beam. The matrix was *m*-nitrobenzyl alcohol. HPLC was performed on a LDC system consisting of a LDC III pump, a UV–VIS detector LDC 1204A with 8 μl flow cell and a 100 μl loop injector. Solvents used for the HPLC experiments were of HPLC grade (Merck). Flash column chromatography (FCC) was performed on Merck silica gel 60 (230–400 mesh) and TLC on Merck silica gel 60 F_{254} films (0.2 mm) precoated on aluminium foil. The solvent systems used were: (A) PhMe–AcOEt (8:2), (B) $CHCl_3$–MeOH (95:5), (C) BuOH–AcOH–H_2O (4:1:1), (D) $CHCl_3$–MeOH–AcOH (85:10:5) and (E) BuOH–AcOH–Py–H_2O (30:6:20:24). Spots were visualized with UV light at 254 nm, with ninhydrin, and chlorine–KI–starch reagent. All solvents were dried and/or purified according to standard procedures[37] prior to use. In particular, THF was distilled from Na–benzophenone. DEAD and TPP were used as purchased (Merck). The coupling agent PyBOP was purchased from Novabiochem and the amino acids from the Protein Research Foundation. (*R*)-Val had a $0.10 \pm 0.04\%$ (*S*)-Val content.

(*R*)-ATHF was prepared from (*S*)-Met according to a literature procedure.[30] In general, the *N*-tosyl derivatives of the amino acids Ala, Ile, GABA, PABA and Lys(Boc) were prepared according to reported general procedures.[3] Similarly, the hydrochlorides of the methyl esters[20] of the amino acids Ala, Ile, Leu and Val, and the tosylates of the benzyl esters[33] of the amino acids Ile and Val were prepared according to well established general procedures. *N*-Tosylation of the thus obtained methyl (AAMs) and benzyl (AABs) esters was uniformly performed as follows. To an ice-cold suspension of HCl·AAM or TosOH·AAB (5 mmol) and TosCl (1 g, 5.25 mmol) in dry $CHCl_3$ (5 ml), was added dropwise Et_3N (1.6 ml, 11.30 mmol) and the resulting reaction mixture was stirred at 0°C for 1–3 h. The solvent was then evaporated off under reduced pressure and the residue partitioned between AcOEt and 5% aqueous citric acid. The organic phase was reextracted with AcOEt and the combined organic layers were washed sequentially with 5% aqueous citric acid, H_2O, twice with 5% aq. $NaHCO_3$ and saturated aq. NaCl (brine) and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure and trituration of the resulting oily residue with hexane provided good yields (85–93%) of pure TAAMs and TAABs as white solids. The thus obtained esters had characteristic sharp IR bands at 3265–3280 (NHTs), 1743–1732 (C = O) and 1345–1340 and 1170–1140 (O = S = O) cm^{-1}. The band at 3265–3280 cm^{-1} disappears, as expected, upon alkylation with the system ROH–TPP–DEAD. For the sake of comparison, and in relation to the change of the acidity of the NHTs group upon its repositioning away from the carboxy group, we would like to note that the N–H bond stretching frequency is at 3235 cm^{-1} for Tos-GABA-OH and at 3277 cm^{-1} for Tos-Ile-OH. Side-chain-protected diamino acids Lys(Tos), Lys(Boc) and Orn(Tos) were prepared according to standard procedures.[38] Boc-Ala, Boc-Lys(Tos), Boc-Orn(Tos) and Cbz-Ala were obtained from Ala and the corresponding derivatives Lys(Tos) and Orn(Tos) using reported general procedures.[39,40] The dipeptide Boc-Orn(Tos)-Ala-OMe was obtained from the corresponding amino and carboxy components by coupling mediated by PyBOP.[31] Dipeptide Tos-Ala-Leu-OMe, tripeptide Tos-Ala-Phe-Leu-OMe and pentapeptide Boc-Lys(Tos)-Gly-Gly-Phe-Leu-OMe were obtained by coupling the corresponding *N*-terminal tosylated amino acids with H-Leu-OMe, H-Phe-Leu-OMe and H-Gly-Gly-Phe-Leu-OMe, respectively, using the mixed anhydride method (^{i}BuOCOCl–NMM).[41] The required amino components, which are actually fragments of the biologically important Leu-enkephalin, were available in our laboratory from other studies.

General procedures for the redox N-*alkylation of* N-*tosylated amino acid and peptide derivatives.* DEAD (0.35 ml, 4.2 mmol) was added dropwise under an atmosphere of argon to an ice-cold solution of TAAM–TAAB–TPM (3.5 mmol), TPP (1.1 g, 4.2 mmol) and ROH (4.2 mmol) in dry THF (10 ml). The resulting solution was kept for 5 min at 0°C and then 15 min at room temperature. In cases where the reaction was not complete (TLC), an additional portion of 2.1 mmol each of TPP, ROH and DEAD was added sequentially at 0°C and after the reaction mixture had been maintained at room temperature for 15 min, the solvent was evaporated off and the oily residue was directly chromatographed (FCC) using PhMe–AcOEt (9:1) as the eluent to afford pure products. For the methylation of TAAs, 2.2 mmol each of TPP, MeOH and DEAD per 1.0 mmol of TAA were used initially followed by an additional portion of 1.1 mmol each of TPP, MeOH and DEAD.

In the methylation of N^{α}-Boc-N^{ω}-tosyldiamino acids (BTAAs), 2.2 mmol each of TPP, MeOH and DEAD per 1.0 mmol of BTAA were initially used and after 30 min at room temperature an additional 1.1 mmol of each of the reagents was added sequentially, and the reaction mixture was stirred at room temperature for 12 h before yet another 1.1 mmol of each of the reagents was added. Finally, after an additional 12 h at room temperature, the resulting reaction mixture was processed as described

above. Methylation of side-chain-tosylated Lys and Orn peptide derivatives was performed as described for BTAAs, but using initially 1.1 mmol of each of the reagents.

Yields, physical characteristics and selected spectral data for fully protected *N*-alkylated derivatives of amino acids and peptides, prepared by the above described procedures, are provided below. Concerning the ^{1}H NMR spectra of these derivatives, the noticable shift of the *N*-Me group towards higher field upon removal of the *N*(Me)Tos function away from the ester function should be pointed out. Thus, the δ value for the *N*-Me group for Boc-Lys(Tos,Me)-OMe is 2.71 ppm whereas that of (Tos,Me)-Lys(Boc)-OMe is 2.84. The aromatic part of the Tos function showed almost identical appearance in all spectra recorded, with respect to chemical shifts and splitting pattern (an AA′XX′ spin system), i.e., two multiplets (deceptive doublets) at δ 7.73–7.62 and 7.34–7.14 ppm.

Tos(Me)-Ala-OMe (**3**). Yield 96%, oil, $[\alpha]_D^{25}$ −52.8° (*c* 0.6, MeOH), R_f(A) = 0.45, R_f(B) = 0.67, Anal. $C_{12}H_{17}NO_4S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 271 (83.3, [*M*]), 269 (7.3, [*M* − H_2]), 211 (100, [*M* − $C_2H_4O_2$]), ^{1}H NMR: δ 4.81 (1 H, q, *J* 7.3 Hz, NC*H*CO_2Me), 3.58 (3 H, s, OCH_3), 2.86 (3 H, s, NCH_3), 2.45 (3 H, s, $ArCH_3$) and 1.36 (3 H, d, *J* 7.3 Hz, $CHCH_3$).

Tos(Me)-Ile-OMe (**6**). Yield 87%, oil, $[\alpha]_D^{25}$ −43.4° (*c* 1, MeOH), R_f(A) = 0.56, R_f(B) = 0.70, Anal. $C_{15}H_{23}NO_4S$: C, H. IR: 1738, 1345 and 1162 cm^{-1}.

Tos(Me)-Leu-OMe (**9**). Yield 89%, m.p. 79–80°C, $[\alpha]_D^{25}$ −35.3° (*c* 1, MeOH), R_f(A) = 0.49, R_f(B) = 0.71, Anal. $C_{15}H_{23}NO_4S$: C, H.

Tos(Me)-Val-OMe (**10**). Yield 89%, m.p. 58–59°C, $[\alpha]_D^{25}$ −37.2° (*c* 1, MeOH), R_f(A) = 0.52, R_f(B) = 0.67, Anal. $C_{14}H_{21}NO_4S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 299 (20, [*M*]), 297 (8.8, [*M* − H_2]), 239 (100, [*M* − $C_2H_4O_2$]), 237 (2.3, [*M* − $C_2H_4O_2$ − H_2]). ^{1}H NMR: δ 4.14 (1 H, d, *J* 10.7 Hz, NC*H*CO_2Me), 3.43 (3 H, s, OCH_3), 2.89 (3 H, s, NCH_3), 2.44 (3 H, s, $ArCH_3$), 2.10 (1 H, m, $CHMe_2$), 1.01 (3 H, d, *J* 6.7 Hz, $CHCH_3$) and 0.94 (3 H, d, *J* 6.6 Hz, $CHCH_3$).

Tos(Me)-GABA-OMe (**20**). Yield 78%, oil, R_f(A) = 0.38, R_f(B) = 0.69, Anal. $C_{13}H_{19}NO_4S$: C, H. IR: 1738, 1345 and 1167 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 285 (100, [*M*]), 253 (77.8, [*M* − MeOH]), 197 (33.3, [TosNCHCH_3]), 129 (66.7, [*M* − TosH]). ^{1}H NMR: δ 3.71 (3 H, s, OCH_3), 3.05 (2 H, t, *J* 6.8 Hz, NCH_2), 2.73 (3 H, s, NCH_3), 2.46 (3 H, s, $ArCH_3$), 2.46 (2 H, t, *J* 7.2 Hz, CH_2CO_2Me) and 1.89 (2 H, quintet, *J* 7.1 Hz, $NCH_2CH_2CH_2CO_2Me$).

Tos(Me)-PABA-OMe (**21**). Yield 85%, m.p. 100–102°C, R_f(A) = 0.52, R_f(B) = 0.70, Anal. $C_{16}H_{17}NO_4S$: C, H. IR: 1721, 1364 and 1176 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 320 (80, [*M*H]), 318 (4.3, [(*M*H) − H_2]), 288 (34.3, [*M*H − CH_3OH]), 165 (100, [*M*H − Tos]), 164 (74.3, [*M* − Tos]).

Tos(Et)-Ala-OMe (**22**). Yield 94%, oil, $[\alpha]_D^{25}$ −28.5°C (*c* 1, MeOH), R_f(A) = 0.48, R_f(B) = 0.66, Anal. $C_{13}H_{19}NO_4S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 285 (53.5, [*M*]), 283 (10, [*M* − H_2]), 225 (100, [*M* − $C_2H_4O_2$]), 129 (10.9, [*M* − TosH]).

Tos(Et)-Ile-OMe (**23**). Yield 82%, oil, $[\alpha]_D^{25}$ −46.0° (*c* 1, MeOH), R_f(A) = 0.58, R_f(B) = 0.71, Anal. $C_{16}H_{25}NO_4S$: C, H. IR: 1743, 1345 and 1151 cm^{-1}.

Tos(Et)-Leu-OMe (**24**). Yield 86%, oil, $[\alpha]_D^{25}$ −63.3° (*c* 1, MeOH), R_f(A) = 0.57, R_f(B) = 0.72, Anal. $C_{16}H_{25}NO_4S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 327 (26.7, [*M*]), 325 (6.6, [*M* − H_2]), 267 (100, [*M* − $C_2H_4O_2$]), 171 (7.5, [*M* − TosH]). ^{1}H NMR: δ 4.62 (1 H, dd, *J* 7.5 and 5.6 Hz, NC*H*CO_2Me), 3.48 (3 H, s, OCH_3), 3.32 [2 H, m (AB part of a ABX_3 spin system), NCH_2CH_3], 2.44 (3 H, s, $ArCH_3$), 1.78 (1 H, m, C*H*Me_2), 1.66 (2 H, m, $CHCH_2CH$), 1.25 (3 H, t, *J* 7.1 Hz, NCH_2CH_3), 1.00 (3 H, d, *J* 4.4 Hz, $CHCH_3$) and 0.97 (3 H, d, *J* 4.6 Hz, $CHCH_3$).

Tos(^{i}Pr)-Ala-OMe (**25**). Yield 87%, oil, $[\alpha]_D^{25}$ −19.2° (*c* 1, MeOH), R_f(A) = 0.47, R_f(B) = 0.67, Anal. $C_{14}H_{21}NO_4S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 299 (48.1, [*M*]), 297 (10.7, [*M* − H_2]), 239 (100, [*M* − $C_2H_4O_2$]), 197 ([$C_9H_{11}O_2SN$]). NMR: δ 4.82 and 4.69 (1 H, two q, *J* 7.3 Hz, NC*H*CO_2Me), 3.58 and 3.57 (3 H, two s, OCH_3), 3.33 (1 H, m, NC*H*Me_2), 2.45 (3 H, s, $ArCH_3$), 1.45 and 1.36 (3 H, two d, *J* 7.3 Hz, $CHCH_3$), 1.23 (3 H, d, *J* 6.7 Hz, $NCHCH_3$) and 1.17 (3 H, d, *J* 6.7 Hz, $NCHCH_3$).

Tos(^{i}Pr)-Ile-OMe (**26**). Yield 79%, oil, $[\alpha]_D^{25}$ −78.3° (*c* 1, MeOH), R_f(A) = 0.59, R_f(B) = 0.72, Anal. $C_{17}H_{27}NO_4S$: C, H. IR: 1743, 1340 and 1151 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 342 ([*M*H]), 282 (68, [(*M* − $C_2H_4O_2$) + H]), 240 ([(*M* − $C_2H_4O_2$) − C_3H_6 + H]), 186 (32, [(*M* − TosH) + H]), 91 ([C_7H_7]).

Tos(Me)-Ile-OBzl (**31**). Yield 85%, oil, $[\alpha]_D^{25}$ −4.5° (*c* 1, MeOH), R_f(A) = 0.63, R_f(B) = 0.74, Anal. $C_{21}H_{27}NO_4S$: C, H. IR: 1738, 1345 and 1151 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 390 (8, [*M*H]), 254 (38, [(*M* − $C_8H_8O_2$) + H]), 91 (100, [C_7H_7]). ^{1}H NMR: δ 7.34 (3 H, m, PhH), 7.18 (2 H, m, PhH), 4.92 and 4.72 (2 H, AB system, *J* 12.3 Hz, OCH_2Ph), 4.31 (1 H, d, *J* 10.7 Hz, NC*H*CO_2Bzl), 2.87 (3 H, s, NCH_3), 2.38 (3 H, s, $ArCH_3$), 1.90 (1 H, m, MeC*H*Et), 1.61 (1 H, m, $CHCH_2CH_3$), 1.18 (1 H, m, $CHCH_2CH_3$), 1.18 (1 H, m, $CHCH_2CH_3$), 0.092 (3 H, t, *J* 7.4 Hz, CH_2CH_3) and 0.86 (3 H, d, *J* 6.6 Hz, CH_3CH).

Tos(Me)-Val-OBzl (**32a**). Yield 88%, m.p. 69–70°C, $[\alpha]_D^{25}$ −40.3° (*c* 1, MeOH), R_f(A) = 0.60, R_f(B) = 0.73, Anal. $C_{20}H_{25}NO_4S$: C, H. IR: 1732, 1340 and 1156 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 375 (91, [*M*]), 239 ([*M* − $C_8H_8O_2$]), 91 (100, [C_7H_7]).

(R)-Tos(Me)-Val-OBzl (**32b**). Yield 87%, m.p. 71°C, $[\alpha]_D^{25}$ +40.3° (*c* 1, MeOH), R_f(A) = 0.60, R_f(B) = 0.73.

Tos(iPr)-Val-OBzl (**33**). Yield 84%, oil, $[\alpha]_D^{25}$ −60.8° (*c* 1, MeOH), R_f(A) = 0.61, R_f(B) = 0.74, Anal. $C_{22}H_{29}NO_4S$: C, H. IR: 1738, 1340 and 1162 cm^{-1}.

Tos(Et)-Ala-Leu-OMe (**36**). Yield 85%, oil, $[\alpha]_D^{25}$ −45.2° (*c* 1, MeOH), R_f(B) = 0.70, R_f(C) = 0.73, Anal. $C_{19}H_{30}N_2O_5S$: C, H. ^{1}H NMR: δ 6.94 (1 H, d, *J* 7.8 Hz, CONH), 4.51 (1 H, m, ^{i}PrCH$_2$C*H*), 4.40 (1 H, q, *J* 7.2 Hz, NHC*H*), 3.74 (3 H, s, OCH_3), 3.44 (1 H, dq, *J* 7.2 and 14.4 Hz, MeCH_2N), 3.20 (1 H, dq, *J* 7.2 and 14.4 Hz, MeCH_2N), 2.44 (3 H, s, $ArCH_3$), 1.66 (3 H, m, CH_2C*H*Me$_2$), 1.17 (3 H, t, *J* 7.2 Hz, NCH$_2$CH_3), 1.08 (3 H, d, *J* 7.2 Hz, CH_3CH), 0.97 (3 H, d, *J* 6.2 Hz, CH$_2$CHCH_3) and 0.94 (3 H, d, *J* 6.1 Hz, CH$_2$CHCH_3).

Tos(Me)-Ala-Phe-Leu-OMe (**37**). Yield 78%, m.p. 130–132°C, $[\alpha]_D^{25}$ −27.5° (*c* 1, MeOH), R_f(B) = 0.66, R_f(C) = 0.76, Anal. $C_{27}H_{37}N_3O_6S$: C, H. IR: 3309, 1743, 1654, 1371 and 1172 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 532 (21.8, [*M*H]), 211 (100, [(Tos + C_3H_6N)]), 90 (21.8, [$C_3H_7NO_2$ + H]). ^{1}H NMR: δ 7.68 [2 H, d, *J* 8.3 Hz, Ar-H (*ortho* to the sulfonamide group)], 7.36–7.17 (7 H, m, ArH and Ph-H), 6.87 (1 H, d, *J* 7.9 Hz, CONH), 6.54 (1 H, d, *J* 8.0 Hz, CONH), 4.67 (1 H, dt, *J* 8.6 and 5.7 Hz, PhCH$_2$C*H*), 4.59 (1 H, m, ^{i}PrCH$_2$C*H*), 4.43 (1 H, q, *J* 7.2 Hz, CH$_3$C*H*), 3.75 (3 H, s, OCH_3), 3.24 (1 H, dd, *J* 14.2 and 5.7 Hz, PhCH_2), 3.05 (1 H, dd, *J* 14.2 and 8.9 Hz, PhCH_2), 2.46 (3 H, s, NCH_3), 2.37 (3 H, s, $ArCH_3$), 1.68–1.52 (3 H, m, CH_2C*H*Me$_2$), 0.95 (3 H, d, *J* 3.6 Hz, CHCH_3), 0.94 (3 H, d, *J* 6.9 Hz, NCHCH_3) and 0.93 (3 H, d, *J* 4.6 Hz, CHCH_3).

Boc-Lys(Tos,Me)-OMe (**51**). Yield 58%, oil, $[\alpha]_D^{25}$ −6.5° (*c* 1, MeOH), R_f(A) = 0.28, R_f(B) = 0.66, Anal. $C_{20}H_{32}N_2O_6S$: C, H. IR: 3361, 1743, 1717, 1340 and 1167 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 429 (1.9, [*M*H]), 373 (16.7, [(*M* − C_4H_8) + H]), 329 (100, [(*M* − $C_5H_9O_2$) + H]), 186 (62, [(TosNHCH$_3$) + H]). ^{1}H NMR: δ 5.07 (1 H, d, *J* 7.0 Hz, NHBoc), 4.35 (1 H, m, NC*H*CO$_2$Me), 3.77 (3 H, s, OCH_3), 2.98 (2 H, m, CH_2NMeTos), 2.71 (3 H, s, NCH_3), 2.45 (3 H, s, $ArCH_3$), 2.00–1.40 [6 H, m, CH(CH_2)$_3$] and 1.47 [9 H, s, C(CH_3)$_3$].

(Tos,Me)-Lys(Boc)-OMe (**53**). Yield 90%, oil, $[\alpha]_D^{25}$ −4.1° (*c* 1, MeOH), R_f(A) = 0.24, R_f(B) = 0.65, Anal. $C_{20}H_{32}N_2O_6S$: C, H. IR: 3319, 1738, 1717, 1345 and 1172 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 429 (8, [*M*H]), 373 (19, [(*M* − C_4H_8) + H]), 329 (100, [(*M* − $C_5H_9O_2$) + H]), 186 (62, [(TsNHCH$_3$) + H]), 57 (57, [C_4H_9]). ^{1}H NMR: δ 4.62 (1 H, dd, *J* 10.2 and 5.5 Hz, NC*H*CO$_2$Me), 4.60 (1 H, br, NHBoc), 3.50 (3 H, s, OCH_3), 3.13 (2 H, m, CH_2NHBoc), 2.84 (3 H, s, NCH_3), 2.45 (3 H, s, $ArCH_3$), 2.00–1.40 [6 H, m, CH(CH_2)$_3$] and 1.48 [9 H, s, C(CH_3)$_3$].

Boc-Lys(Tos,Me)-Gly-Gly-Phe-Leu-OMe (**55**). Yield 55%, m.p. 117–119°C, $[\alpha]_D^{25}$ −52.0° (*c* 0.5, MeOH), R_f(B) = 0.22, R_f(C) = 0.75, Anal. $C_{39}H_{58}N_6O_{10}S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 803 ([*M*H]).

Boc-Orn(Tos,Me)-OMe (**58**). Yield 60%, oil, $[\alpha]_D^{25}$ −12.7° (*c* 0.7, MeOH), R_f(A) = 0.27, R_f(B) = 0.66, Anal. $C_{19}H_{30}N_2O_6S$: C, H. IR: 3372, 1743, 1712, 1340 and 1167 cm^{-1}.

Boc-Orn(Tos,Me)-Ala-OMe (**61**). Yield 70%, oil, $[\alpha]_D^{25}$ −14.2° (*c* 1, MeOH), R_f(B) = 0.58, R_f(C) = 0.74, Anal. $C_{22}H_{35}N_3O_7S$: C, H. FAB-MS [*m/z* (% rel. int.)]: 485 (6.7, [*M*]), 385 (100, [*M* − Boc]), 254 (42.6, [(*M* − Boc) − (Ala-OMe) − CO_2]), 198 (60, [TosN(Me) = CH_2]).

(S) and (R)-N-Methyl-N-(4-toluenesulphonyl)valine (R)-3-tetrahydrofurylamide (**27** *and* **28**). *Carboxy-deprotection by hydrogenolysis.* To a solution of (*S*)-(**32a**) or (*R*)-(**32b**) benzyl ester (1.08 g, 3 mmol) in glacial AcOH (15 ml) were added 0.2 g of 10% Pd-on-C and two drops of 70% perchloric acid and through the resulting, vigorously stirred, suspension, hydrogen gas was gently bubbled for 5 h at room temperature. The catalyst was then filtered off through a short Celite bed, washed with glacial AcOH and the filtrate was concentrated to a small volume under reduced pressure and diluted with 50 ml of water. The product was extracted with AcOEt (2 × 30 ml), the combined organic layers were washed with brine (3 × 30 ml), dried and evaporated to afford 0.81 g (95%) and 0.79 g (93%), respectively, of pure (TLC) enantiomeric acids (Tos,Me)-Val-OH as white powders with R_f(C) = 0.74.

Carboxy-deprotection by saponification. To an ice-cold solution of methyl ester **10** (0.5 g, 1.67 mmol) in MeOH (5 ml), was added dropwise 2 M NaOH (1.7 ml). The resulting reaction mixture was stirred at room temperature for 4 h and additional 2 M NaOH (0.8 ml) was added at 0°C and further stirred at room temperature for 12 h. The MeOH was subsequently evaporated off under reduced pressure, the residue was diluted with H_2O and the resulting solution was ice-cooled and brought to pH 2 by the dropwise addition of 6 M HCl. Extraction with AcOEt (3 × 30 ml), washing of the combined extracts with brine, drying and evaporation afforded 0.44 g (93%) of pure (TLC) product.

Carboxy-deprotection by TMSI mediated splitting. To a solution of methyl ester **10** (0.3 g, 1.02 mmol) in dry $CHCl_3$

(4 ml), was added TMSI (0.29 ml, 2 mmol) and the resulting solution was refluxed with exclusion of moisture for 30 h. Evaporation under reduced pressure followed by FCC of the residue using $CHCl_3$–MeOH (9:1) as the eluent provided 0.27 g (90%) of pure (TLC) product.

PyBOP-mediated coupling with (R)-ATHF. To an ice-cold mixture of (*S*)-(Tos,Me)-Val-OH (0.28 g, 1 mmol), as obtained from **32a** through hydrogenolysis, TosOH·ATHF (0.29 g, 1.1 mmol) and PyBOP (0.69 g, 1 mmol) in anhydrous CH_2Cl_2 (1 ml), was added diisopropylethylamine (DIEA) (3 mmol) and the resulting reaction mixture was stirred at 0 °C for 1 min and at room temperature for 1 h. The solvent was then evaporated off under reduced pressure and the residue was partitioned between 5% aqueous citric acid and EtOAc. The aqueous layer was reextracted with EtOAc and the combined organic layers were washed sequentially with H_2O, 5% aq. $NaHCO_3$ and brine, dried and evaporated to leave an oily residue. FCC using PhMe–EtOAc (1:1) as the eluent provided 0.28 g of amide **27** as a white solid. Amide **27** had m.p. 105–107 °C, $[\alpha]_D^{25}$ +21.8° (*c* 0.6, MeOH), R_f(B) = 0.54, R_f(C) = 0.71. Anal. $C_{17}H_{26}N_2O_4S$: C, H. IR: 3372, 3214, 1670, 1340 and 1162 cm^{-1}. FAB-MS [*m/z* (% rel. int.)]: 355 (82, [*M*H]), 353 (13, [(*M*H) – H_2]), 240 (100, [(*M* – $C_5H_9NO_2$)H]), 86 (53, [C_4H_8NO]).

All other samples of (Tos,Me)-Val-ATHF were obtained under identical reaction conditions with those described above. However, coupling of (*R*)-(Tos,Me)-Val-OH to TosOH·ATHF required several hours at room temperature to go to completion. It should be mentioned that **27** and **28** had identical R_f values (TLC) for various solvent systems tested. For the sake of brevity, the thus obtained four samples of (Tos,Me)-Val-ATHF are designated as: A, obtained through hydrogenolysis of **32a**; B, obtained through hydrogenolysis of **32b**; C, obtained through saponification of **10**; D, obtained through TMSI splitting of **10**. Samples A–D were examined by HPLC under the following experimental conditions. The analytical column used was a Lichrosorb SI-100, 5 μm particle size, 250 × 4.6 mm ID. The column was packed by the slurry technique in a Magnus slurry-packing unit P6060 with *n*-heptane, at a pressure of 8000 psi. Elution was performed at 1.2 ml min^{-1} with hexane–AcOEt–MeOH (40:6:1) and detection at 260 nm. The results of these experiments are presented in Fig. 4. It is thus evident that samples A and D represent the pure diastereomer **27**, whereas samples B and C are mixtures of diastereomers **27** and **28**.

Deprotection of fully protected N-alkyl-N-tosylamino acids and peptides. In general, AAAs were obtained from the corresponding TAAABs (0.5 g scale) through hydrogenolysis as described above, followed by routine detosylation with Na in liquid NH_3 as described in ref. 36. AAA amides were derived from the corresponding TAAAMs (0.5 g scale) through ammonolysis followed by detosylation. APs and AP amides were obtained from the corresponding TAPMs (0.3 g scale) through saponification and ammonolysis,[42] respectively, followed by detosylation. Work-up of the reaction mixtures from Na–liquid NH_3-mediated detosylation was carried out by careful addition of glacial AcOH followed by slow evaporation of ammonia. The residue was dissolved in MeOH and the resulting solution was evaporated to dryness and this treatment was repeated. In the case of Me-Ala-NH_2 (**40**), the resulting solid residue was leached several times with Et_2O and the organic phase was evaporated to leave the pure product, whereas in the case of tripeptide **42** the resulting solid residue was dissolved in the minimum amount of water and refrigerated. The precipitated product was then filtered, washed with ice-cold water and dried over P_2O_5 to provide pure **42**. For the rest of the compounds mentioned below the residue was dissolved in the minimum amount of H_2O and the pH was adjusted where necessary to ca. 6 by addition of glacial AcOH. The thus obtained solution was directly subjected to the following purification procedure. The crude products were invariably subjected to reversed-phase FCC (RP-FCC), using water as the eluent, to remove salts and minor by-products formed during detosylation of peptide derivatives. RP-FCC was performed using reversed-phase silica prepared as described in a published procedure.[43] The column used was 7 cm length and 1.7 cm ID and was packed using a slurry of 15 g of RP-silica gel in distilled water and by application of a gentle pressure on the top of the column. After application of the sample dissolved in the minimum amount of water, the column was eluted with distilled water and fractions of 0.5 ml volume were collected. The ninhydrin-active fractions were combined and freeze–dried to afford pure products as white solids. The purity of all products thus purified was verified by RP-HPLC and ion-exchange HPLC (IC-HPLC) under the following experimental conditions. The analytical column used for RP was a Supelcosil LC-18, 5 μm particle size, 250 × 4.6 mm ID, purchased from Supelco, equipped with a RP-18 guard column, 30 × 4.6 mm ID, purchased from Brownlee Labs. The eluted peaks were recorded at 231 nm. Elution was carried out at 1.0 ml min^{-1} with distilled water. The analytical column used for IC-HPLC was an Econosphere-NH_2 5U, purchased from Alltech, 250 × 4.6 mm ID, equipped with an amino guard column (30 × 2.1 mm ID), also purchased from Alltech. Elution was performed with a 30% solution of acetonitrile in 50 mM acetate buffer pH 5.5. The flow rate was 1.5 ml min^{-1} and detection was at 215 nm. As expected, AAA and AP amides were not retained by this column. Eluents used in these HPLC experiments were degassed by vacuum filtration through a 0.2 μm membrane filter followed by agitation in an ultrasonic bath.

*Me-Ile-OH (**38**).* Yield 76%, m.p. 258–260 °C (decomp.), $[\alpha]_D^{25}$ +28.4° (*c* 0.5, H_2O), R_f(C) = 0.33, R_f(E) = 0.42, t_R(RP) = 15.5 min, t_R(IC) = 14.5 min. For comparison, the corresponding t_R values for H-Ile-OH were 9.8 and 7.2 min, respectively. FAB-MS [*m/z* (% rel. int.)]: 146

(100, [*M*H]), 102 (44.2, [*M*H – CO_2]), 100 (32.6, [*M*H – HCO_2H]), 89 (48.8, [*M*H – C_4H_9]). ^{1}H NMR: δ 3.36 (1 H, d, *J* 4.1 Hz, NC*H*CO_2H), 2.58 (3 H, s, NCH_3), 1.82 (1 H, m, C*H*CH_3), 1.41 and 1.17 (2 H, two m, CH_2CH_3), 0.85 (3 H, d, *J* 7.0 Hz, CHCH_3) and 0.82 (3 H, t, *J* 7.3 Hz, CHCH_3).

^{i}Pr-Val-OH (**39**). Yield 72%, m.p. 245°C (decomp.), $[\alpha]_D^{25}$ +23.5° (*c* 1, H_2O), R_f(C) = 0.32, R_f(E) = 0.40, t_R(RP) = 17.0 min, t_R(IC) = 15.0 min. For comparison, the corresponding t_R values for H-Val-OH were 8.0 and 6.5 min, respectively. IR: 3445, 3099–2190, 1569 and 1096 cm^{-1}. FAB-MS [*m*/*z* (% rel. int.)]: 160 (100, [*M*H]), 114 (19.2, [*M*H – HCO_2]), 72 (48.5, [Me_2CHNCH_2]. ^{1}H NMR: δ 3.42 (1 H, d, *J* 4.4 Hz, NC*H*CO_2H), 3.27 (1 H, m, NC*H*), 2.09 (1 H, m, CHC*H*Me_2), 1.22 (3 H, d, *J* 6.6 Hz, NCHCH_3), 1.15 (3 H, d, *J* 6.6 Hz, NCHCH_3), 0.92 (3 H, d, *J* 4.4 Hz, CHCHCH_3) and 0.88 (3 H, d, *J* 4.4 Hz, CHCHCH_3).

Me-Ala-NH_2 (**40**). Yield 72%, m.p. 122–124°C, $[\alpha]_D^{25}$ –13.7° (*c* 1, MeOH), R_f(C) = 0.26, R_f(E) = 0.30, t_R(RP) = 4.0 min. IR: 3277, 3057 and 1685 cm^{-1}. FAB-MS [*m*/*z* (% rel. int.)]: 103 (100, [*M*H]), 89 (21.3, [*M*H – Me]), 58 (66.7, [*M*H – $HCONH_2$]).

Et-Ala-Leu-NH_2 (**41**). Yield 80%, m.p. 238°C (decomp.), $[\alpha]_D^{25}$ –76.8° (*c* 0.25, MeOH), R_f(C) = 0.38, R_f(E) = 0.45, t_R(RP) = 8.5 min. FAB-MS [*m*/*z* (% rel. int.)]: 230 (100, [*M*H]), 185 (3.6, [*M*H – $HCONH_2$]), 88 (16.4, [H-Ala-NH_2]), 71 (57.6, [$EtNCHCH_3$]).

Me-Ala-Phe-Leu-OH (**42**). Yield 74%, m.p. 210°C (decomp.), $[\alpha]_D^{25}$ –27.5° (*c* 0.25, DMF), R_f(C) = 0.52, R_f(E) = 0.58, t_R(RP) = 27.0 min, t_R(IC) = 8.0 min. FAB-MS [*m*/*z* (% rel. int.)]: 364 (100, [*M*H]), 279 (20, [*M*H – MeNCMeCO]), 233 (7.3, [(279 – CO_2 – H_2]), 205 (21.8, [233 – CO]), 120 (90.9, [(Ph–CH = CH–NH_2) + H]).

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