Pergamon

Tetrahedron 56 (2000) 309–315

TETRAHEDRON

Synthesis of (±)-Anatoxin-*a* and Analogues

Philip J. Parsons,* Nicholas P. Camp, Neil Edwards and L. Ravi Sumoreeah

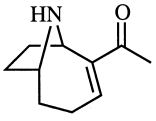
The Chemical Laboratories, School of Chemistry, Physics & Environmental Science, Arundel Building, University of Sussex, Falmer, Brighton BN1 9QJ, UK

Received 28 January 1999; revised 22 September 1999; accepted 7 October 1999

Abstract—A new and highly efficient synthesis of the potent nicotinic acetylcholine receptor agonist, anatoxin-*a* and its analogues is described, which uses a β-lactam ring opening–transannular cyclisation sequence to set up the bridged bicyclic framework of the natural product. The synthesis involves a cycloaddition of chlorosulfonyl isocyanate with cyclooctadiene followed by Boc protection of the resulting β-lactam. Reaction of the β-lactam with a variety of nucleophiles, followed by selenium-mediated cyclisation and oxidation gave the skeleton of anatoxin-*a* bearing various sidechains. The approach offers a flexible entry to useful quantities of anatoxin-*a* and its analogues.

Introduction

Since its isolation and characterisation in the 1970s,[1,2] anatoxin-*a* (**1**) has stimulated the scientific community worldwide. Whilst synthetic chemists devised methods for the construction of the novel 9-azabicyclo[4.2.1]nonane ring system, medicinal chemists were intrigued by its powerful biological activity. A recent review highlights the various syntheses of this molecule, in both optically pure and racemic form, achieved over the last 20 years.[3]



1 Anatoxin-a

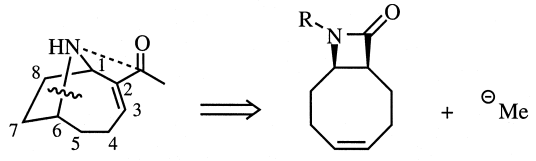
Ingestion of pond water, containing this low molecular weight alkaloid has proven fatal to wildlife, resulting in many deaths.[4] It has been deduced that anatoxin-*a* mimics the neurotransmitter acetylcholine and therefore acts as a potent agonist for the nicotinic acetylcholine receptor nAChR.[5] Due to its resistance to degradation by the enzyme acetylcholine esterase, anatoxin-*a* remains available to overstimulate muscle tissue, resulting in respiratory paralysis and ultimately death. However, despite its poisonous nature, anatoxin-*a* has become a useful pharmacological probe, providing information with regard to acetylcholine-mediated neurotransmission. The Rapoport group has been the leading light in the field providing many analogues to study the binding requirements of the nAChR.[6–8] Gallagher and his co-workers have also made major contributions to this area.[9,10]

As acetylcholine deficiency is implicated in disease states such as Alzheimer's it is hoped that analogues of anatoxin-*a*, possessing lower levels of toxicity, may be used as acetylcholine replacements in the treatment of brain disorders. With this aim in mind, we now wish to report our improved synthesis of (±)-anatoxin-*a*, which can be applied to the synthesis of anatoxin analogues by introducing sidechains other than methyl at an early stage.

Results and Discussion

Our retrosynthetic analysis of anatoxin-*a* (**1**) recognised the connection between the bridging nitrogen atom and the methyl ketone carbonyl. A further disconnection between the bridging nitrogen and C-6 would result in β-lactam (**2**), which could be easily accessed from 1,5-cyclooctadiene (Scheme 1).

Our key idea involved a tandem methyllithium induced β-lactam ring opening reaction, with concomitant intramolecular trapping of the resultant nitrogen anion onto the

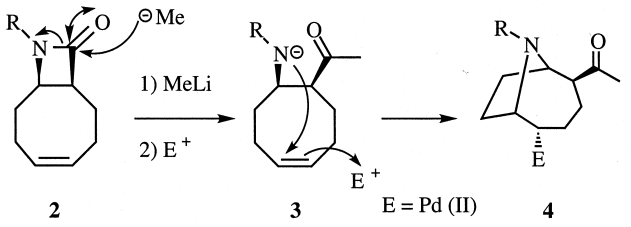


1 **2**

Scheme 1.

Keywords: (±)-anatoxin-*a*; β-lactam; transannular cyclisation.
* Corresponding author. Tel.: +44-1273-678-861; fax: +44-1273-677-196; e-mail: p.j.parsons@sussex.ac.uk

0040–4020/00/$ - see front matter
PII: S0040-4020(99)00909-6



Scheme 2.

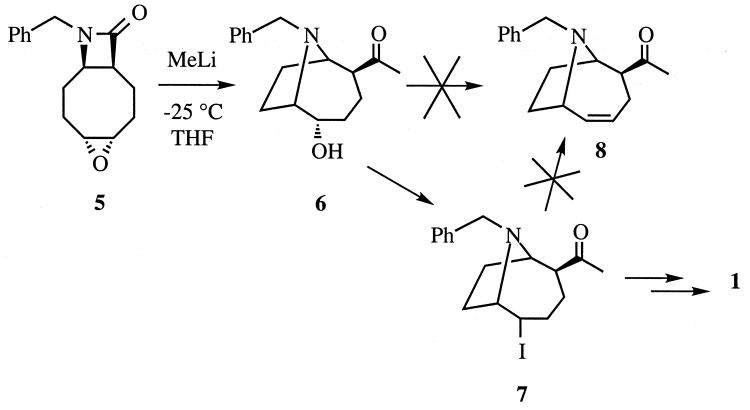
alkene, in the presence of a suitable electrophile (E^+) (Scheme 2).

Potentially we could introduce the methyl ketone at the correct oxidation level and create the desired ring system in one synthetic operation. Additionally, if the tandem reaction could be achieved in the presence of Pd(II), double bond isomerisation towards the conjugated system of anatoxin-*a* could be achieved. Although our early efforts using Pd(II) alkene activation were unsuccessful, a recent publication demonstrated a palladium-mediated transannular cyclisation.[11] The β-lactam epoxide (**5**) was synthesised, and we successfully achieved the desired tandem reaction to produce the bicyclic alcohol (**6**) in a moderate 40% yield (Scheme 3). Attempts to dehydrate the alcohol (**6**) failed, although conversion into the corresponding iodide was achieved in good yield. To our great disappointment, elimination of the iodide also proved fruitless, with alkene (**8**) remaining elusive to us. The iodide (**7**) was reduced using tributyltin hydride and the natural product was completed[12,13] using the chemistry developed by Rapoport.[14]

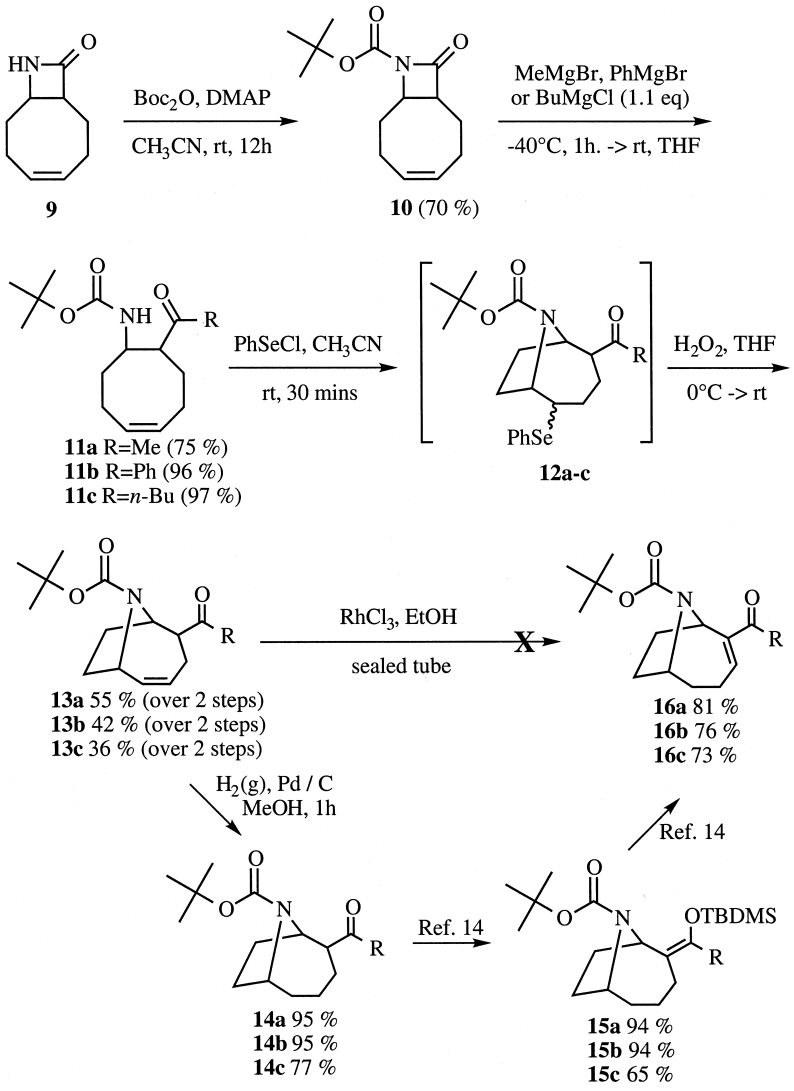
We now wish to report an improved synthesis of (±)-anatoxin-*a*, which allows access to analogues in useful quantities and which also facilitated the synthesis of the postulated key intermediate, alkene (**8**), inaccessible via Scheme 3. Due to the moderate yield of the tandem β-lactam/epoxide opening and the problems encountered with dehydration and elimination, we decided upon a stepwise approach (Scheme 4).

Treatment of β-lactam (**9**)[13] in acetonitrile with $(Boc)_2O$, and DMAP[15] afforded the Boc-protected β-lactam (**10**) in 70% yield. We reasoned that the urethane protecting group should further increase the electrophilic nature of the β-lactam carbonyl. To our delight, treatment of the Boc-protected β-lactam with methylmagnesium bromide in THF at −40°C for 2 h provided the methyl ketone (**11a**) in 75% yield as a white solid.[16] Only small quantities of over-addition products were observed with the remaining 20% unreacted starting material. With the methyl ketone (**11a**) in hand, we looked at several methods for the urethane alkene cyclisation. As described earlier, we attempted to activate the alkene with $PdCl_2$, $Pd(OAc)_2$ and $RhCl_3$. However, under various conditions, the desired cyclisation of the urethane nitrogen onto the alkene failed; instead we observed isomerisation of the alkene. Organoselenium-induced cyclisations of olefinic urethanes are well documented[17–19] and offered us a route to alkene (**13**) via oxidation and elimination of the intermediate selenide. Treatment of the methyl ketone (**11a**) in acetonitrile with 1 equiv. of phenylselenenyl chloride gave a spot-to-spot conversion by TLC to the selenide (**12a**) within 30 min.[20] The crude selenide in THF was treated with aqueous H_2O_2 in the presence of $NaHCO_3$. The resulting selenoxide was left to stir overnight in the presence of triethylamine, to afford the alkene (**13a**) in 55% yield after purification. Purification of the selenide (**12a**) by flash chromatography prior to oxidation gave an improved 71% yield of the alkene.

Using the method of Grieco[21] we attempted to isomerise the alkene (**13a**) using Rh(III) catalysis, to provide Boc-protected anatoxin (**16a**). In accordance with the report of Rapoport[22] we observed a mixture of three alkenes with increased reaction times and the addition of various bases failing to drive the reaction to completion. To overcome this, the alkene was hydrogenated in excellent yield to provide Boc-dihydroanatoxin (**14a**). Introduction of the conjugated double bond was achieved using the methodology developed by the Rapoport group.[14] This general route was also applicable to the synthesis of analogues of anatoxin-*a*, although minor modifications were required. In the case of the *n*-butyl analogue, β-lactam (**10**) could only be ring-opened efficiently by treatment with *n*-butylmagnesium chloride, since the corresponding bromide led to over-addition products. The completion of the synthesis was carried out as described for anatoxin itself. The phenyl analogue presented no problems. In the event, the route was carried out smoothly and served to highlight the



Scheme 3.



Scheme 4.

generality of this approach for the synthesis of C-10 modified anatoxin analogues.

Experimental

Reactions were conducted in flame-dried or oven-dried (175°C, overnight) glassware, under a dry nitrogen atmosphere except when noted otherwise. Solvents and reagents were freshly distilled as follows: tetrahydrofuran (THF) was distilled from sodium/benzophenone; dichloromethane, acetonitrile, and triethylamine were distilled from CaH_2. Analytical thin layer chromatography (TLC) was performed on Merck glass-backed thin layer chromatography plates pre-coated with a 0.25 mm layer of 60 F_{254} silica gel containing a fluorescent indicator. Visualisation was achieved by ultraviolet light (254 nm), iodine or by staining with alkaline potassium permanganate solution, phosphomolybdic acid solution (5% in ethanol) or acidic ceric ammonium nitrate, followed by heating. Evaporation under reduced pressure was achieved using a Büchi rotary evaporator, using water aspirator pressure. Flash column chromatography was carried out using Merck Kieselgel 60 silica gel (Merck art. no. 9385, 230–400 mesh, 0.04–0.063 nm). Melting points (electrothermal apparatus, open capillary) are uncorrected. NMR spectra were recorded in $CDCl_3$, unless otherwise stated, at 300 MHz (^1H) on a Bruker Advance AC-300 instrument and at 75.5 MHz (^{13}C) on the same instrument, and chemical shifts are recorded in parts per million (δ) downfield from Me_4Si (^1H) or relative to $CDCl_3$ (central line of triplet at 77.0 ppm) (^{13}C). ^1H NMR data are tabulated in order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), coupling constant(s) in Hertz (Hz), number of protons. In cases where DEPT experiments were undertaken with ^{13}C NMR acquisitions, the carbon multiplicities are listed as (0) quaternary; (1) methine; (2) methylene; (3) methyl. Both ^1H and ^{13}C NMR spectra of some of the Boc-protected intermediates are complicated by the presence of carbamate rotamers.

3-(*tert*-Butoxycarbonyl)-3-azabicyclo[6.2.0]dec-7-ene-2-one (10). A 500 mL two-necked round-bottomed flask, equipped with a nitrogen inlet adaptor, was charged with

β-lactam **9** (13.1 g, 87 mmol), di-*tert*-butyl dicarbonate (37.8 g, 174 mmol), and DMAP (1.07 g, 8.7 mmol) in acetonitrile (200 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and left to stir overnight. The solvent was removed under reduced pressure and the residue dissolved in dichloromethane (200 mL) and washed with $NaHSO_3$ (2×50 mL), saturated $NaHCO_3$ (1×50 mL), and brine (1×50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to afford a yellow oil. Purification using flash column chromatography on silica gel (elution with 3:7 ether–petroleum ether) provided **10** as a white solid (15.2 g, 70%): mp 82–84°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.42; IR (CH_2Cl_2) 1800, 1717 cm^{-1}; ^1H NMR δ 1.50 (s, 9H, Boc), 1.85–2.00 (m, 1H, alkyl), 2.02–2.18 (m, 4H, alkyl), 2.35–2.57 (m, 3H, alkyl), 3.27–3.38 (m, 1H, C*H*C=O), 4.06–4.15 (m, 1H, C*H*NBoc), 5.64–5.79 (m, 2H, olefinic); ^{13}C NMR δ 24.41 (2), 25.56 (2), 26.13 (2), 29.77 (2), 30.02 (3), 54.71 (1), 58.21 (1), 84.85 (0), 132.46 (1), 132.72 (1), 150.08 (0), 170.28 (0); CIMS *m*/*z* (relative intensity) 269 ($MH^+ + NH_3$, 29), 252 (MH^+, 20), 213 ($MH^+ - (H_3C)_2CCH_2 + NH_3$, 65), 196 ($MH^+ - (H_3C)_2CCH_2$, 100); HRMS, *m*/*z* calcd for $C_{10}H_{13}NO_3$ ($M^+ - (H_3C)_2CCH_2$) 195.0895, found 195.0897; Anal. Calcd for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.94; H, 8.46; N, 5.54.

***cis*-5-Acetyl-6-([*tert*-butoxycarbonyl]amino)cyclooctene (11a).** This procedure constitutes a modification to the method described by Nozoe and co-workers.[16] A 500 mL two-necked round-bottomed flask, equipped with a nitrogen inlet adaptor, was charged with Boc protected β-lactam **10** (11.73 g, 47 mmol), in THF (200 mL) and cooled to −40°C. Methylmagnesium bromide (3 M in ether, 17 mL, 51 mmol) was added dropwise to the solution and the reaction mixture was left to stir at −40°C for 2 h. After this time, the reaction was quenched with saturated NH_4Cl solution (20 mL) and poured into ether (200 mL).The organic layer was washed with H_2O (1×50 mL), brine (1×50 mL), dried over $MgSO_4$, filtered, and concentrated to afford a colourless oil. Purification using flash column chromatography on silica gel (elution with 2:3 ether–petroleum ether) provided **11a** as a white solid (9.36 g, 75%): mp 83–85°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.34; IR (CH_2Cl_2) 3442, 1708, 1704 cm^{-1}; ^1H NMR δ 1.43 (s, 9H, Boc), 1.75–1.87 (m, 2H, alkyl), 1.90–2.22 (m, 4H, alkyl), 2.19 (s, 3H, methyl), 2.25–2.48 (m, 2H, alkyl), 2.75–2.86 (m, 1H, C*H*C=O), 4.16–4.28 (m, 1H, C*H*NBoc), 4.81–4.92 (br, 1H, N*H*), 5.59–5.80 (m, 2H, olefinic); ^{13}C NMR δ 24.26 (2), 25.44 (2), 26.40 (2), 28.76 (3), 29.20 (3), 33.75 (2), 50.25 (1), 54.24 (1), 79.83 (0), 129.71 (1), 130.89 (1), 155.55 (0), 211.0 (0); FAB *m*/*z* (relative intensity) 290 ($M+Na^+$, 10), 268 (MH^+, 42), 212 ($MH^+ - (H_3C)_2CCH_2$, 85), 168 (MH^+−Boc, 100); HRMS, *m*/*z* calcd for $C_{15}H_{25}NO_3$ (M^+) 267.1834, found 267.1814; Anal. Calcd for $C_{15}H_{25}NO_3$: C, 67.38; H, 9.42; N, 5.24. Found: C, 67.31; H, 9.58; N, 5.22.

***cis*-5-Benzoyl-6-([*tert*-butoxycarbonyl]amino)cyclooctene (11b).** Prepared according to the general procedure via addition of phenylmagnesium bromide to β-lactam (**10**). Purification was carried out by column chromatography on silica gel (elution with 3:7 ether–petroleum ether) affording the title compound as a white crystalline solid (96%): mp 127–130°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.52; IR ($CHCl_3$) 2932, 1698 cm^{-1}; ^1H NMR δ 1.32 (s, 9H, Boc), 1.62–1.77 (m, 2H, alkyl), 1.88–2.28 (m, 4H, alkyl), 2.33–2.63 (m, 2H, alkyl), 3.87 (br s, 1H, C*H*C=O), 4.12–4.24 (m, 1H, C*H*NBoc), 4.54–4.69 (m, 1H, N*H*), 5.58–5.79 (m, 2H, olefinic), 7.33–7.53 (m, 3H, aromatic), 7.80 (d, J=7.9 Hz, 2H, aromatic) ppm; ^{13}C NMR δ 23.90 (2), 26.70 (2), 28.74 (3), 33.46 (2), 47.45 (1), 50.41 (0), 79.73 (0), 126.28 (2), 128.70 (1), 129.07 (1), 130.13 (1), 130.40 (1), 133.27 (1), 147.97 (0), 155.45 (0) ppm; FABMS *m*/*z* (relative intensity) 352 ($M+Na^+$, 6), 330 (MH^+, 20), 274 ($MH^+ - (H_3C)_2CCH_2$, 38), 230 (MH^+−Boc, 82); HRMS *m*/*z* calcd for $C_{16}H_{19}NO_3$ ($M^+ - (H_3C)_2CCH_2$) 273.1365, found 273.1365.

***cis*-5-Valeryl-6-([*tert*-butoxycarbonyl]amino)cyclooctene (11c).** Prepared by the general method described above, via addition of *n*-butylmagnesium chloride to β-lactam (**10**) and stirring at −40°C for 1 h. Purification using flash column chromatography on silica gel (elution with 2:3 ether–petroleum ether) afforded the title compound as a colourless oil (3.23 g, 97%): TLC (2:3 ether–petroleum ether) R_f 0.53; IR (film) 2933, 1699 cm^{-1}; ^1H NMR δ 0.65–0.73 (m, 3H, butyl), 1.01–1.16 (m, 2H, butyl), 1.20 (s, 9H, Boc), 1.29–1.40 (m, 2H, alkyl), 1.50–1.66 (m, 2H, alkyl), 1.76–2.08 (m, 4H, alkyl), 2.10–2.39 (m, 4H, alkyl+butyl), 2.60–2.70 (m, 1H, C*H*C=O), 3.90–4.03 (m, 1H, C*H*NBoc), 4.57–4.69 (m, 1H, N*H*), 5.40–5.60 (m, 2H, olefinic); ^{13}C NMR δ 13.74 (2), 22.17 (2), 22.45 (2), 25.17 (2), 28.87 (3), 33.08 (2), 40.97 (1), 52.81 (1), 79.18 (0), 129.12 (1), 130.06 (1), 154.91 (0), 212.77 (0) ppm; EIMS *m*/*z* (relative intensity) 309 (M^+, 2), 253 ($M^+ - (H_3C)_2CCH_2$, 65), 209 (M^+−Boc, 50); HRMS, *m*/*z* calcd for $C_{18}H_{31}NO_3$ (M^+) 309.2304, found 309.2312.

2-Acetyl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-non-4-ene (13a). A 500 mL two-necked round-bottomed flask, equipped with a nitrogen inlet adaptor, was charged with methyl ketone **11** (7.8 g, 29 mmol), in acetonitrile (250 mL). Phenylselenenyl chloride (5.60 g, 29 mmol) was added portionwise to the solution and the reaction mixture was left to stir at room temperature for 30 min. After this time, the solvent was removed and the residue taken up in ether (200 mL). The organic layer was washed with saturated $NaHCO_3$ (2×50 mL), dried over $MgSO_4$, filtered, and concentrated to afford the selenide **12a** as a dark yellow oil (11.83 g, 96%).

Oxidative elimination: To a solution of the crude selenide **12a** (11.83 g, 28 mmol) in THF (200 mL) was added $NaHCO_3$ (4.7 g, 56 mmol) and 27% aqueous H_2O_2 (35.3 g, 280 mmol) dropwise at 0°C. After stirring at room temperature for 1 h, triethylamine (8.6 mL, 62 mmol) was added and the reaction mixture left to stir overnight. After this time, the reaction mixture was poured into ether (200 mL), washed with saturated $NaHCO_3$ (2×50 mL), dried over $MgSO_4$, filtered, and concentrated to yield a colourless oil. Purification using flash column chromatography on silica gel (elution with 2:3 ether–petroleum ether) provided **13a** as a white crystalline solid (4.1 g, 55%): mp 88–90°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.27; IR (CH_2Cl_2) 1712, 1691 cm^{-1}; ^1H NMR δ 1.45 (s, 9H, Boc), 1.60–2.30 (m,

4H, alkyl), 2.24 and 2.30 (s, 3H, methyl), 2.41–2.58 (m, 2H, alkyl), 2.62–2.74 (m, 1H, alkyl), 4.48–4.59 (m, 1H, bridgehead), 4.60–4.78 (m, 1H, bridgehead), 5.56–5.70 (m, 2H, olefinic); ^{13}C NMR (two rotamers 4:10) δ 24.18, 24.96 (2), 27.98, 28.20 (3), 28.26, 28.32 (3), 29.52, 30.44 (2), 32.07, 33.86 (2), 54.77, 55.34 (1), 57.33, 57.67 (1), 61.49, 62.50 (1), 79.40, 80.23 (0), 125.39, 126.11 (1), 132.24, 133.46 (1), 153.28, 153.84 (0), 207.63, 208.8 (0); EIMS *m/z* (relative intensity) 265 (M^+, 13), 209 ($M^+-(H_3C)_2CCH_2$, 32), 192 ($M^+-(H_3C)_2CCH-H_2O$, 35), 165 (M^+-Boc, 48); HRMS, *m/z* calcd for $C_{15}H_{23}NO_3$ (M^+) 265.1670, found 265.1678; Anal. Calcd for $C_{15}H_{23}NO_3$: C, 67.90; H, 8.74; N, 5.28. Found: C, 67.81; H, 8.80; N, 5.13.

2-Benzoyl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-non-4-ene (13b). Prepared according to the general procedure. Purification was carried out using flash column chromatography (elution with 2:3 ether–petroleum ether) providing the title compound as a white crystalline solid (3.38 g, 42%): mp 113–116°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.44; IR ($CHCl_3$) 2973, 1695 cm^{-1}; ^{1}H NMR δ 1.37, 1.43 (s, 9H, Boc), 1.64–1.91 (m, 2H, alkyl), 2.06–2.23 (m, 2H, alkyl), 2.57–2.80 (m, 2H, alkyl), 3.60, 3.63 (s, 1H, alkyl), 4.31–4.82 (m, 2H, bridgeheads), 5.52–5.80 (m, 2H, olefinic), 7.38–7.63 (m, 3H, aromatic), 7.87 (d, *J*=7.4 Hz, 2H, aromatic) ppm; ^{13}C NMR δ 24.30 (2), 28.48, 28.73 (3), 29.92 (2), 34.70 (2), 55.75 (1), 58.05 (1), 58.69 (1), 80.34 (0), 126.37 (1), 128.76, 128.88, 128.99, 129.07 (1), 131.99, 133.17 (1), 136.34 (0), 153.67 (0), 199.66 (0) ppm; EIMS *m/z* (relative intensity) 327 (M^+, 37), 271 ($M^+-(H_3C)_2CCH_2$, 24), 254 ($M^+-(H_3C)_2CCH-H_2O$, 16), 227 (M^+-Boc, 64); HRMS calcd for $C_{16}H_{17}NO_3$ ($M^+-(H_3C)_2CCH_2$) 271.1208, found 271.1212.

2-Valeryl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-non-4-ene (13c). Prepared according to the general procedure. Purification was carried out using flash column chromatography (elution with 3:1 petroleum ether–ether) providing the title compound as a colourless oil (366 mg, 36%): TLC (3:1 petroleum ether–ether) R_f 0.16; IR (film) 2961, 1696 cm^{-1}; ^{1}H NMR δ 0.78–0.90 (m, 3H, butyl), 1.13–1.31 (m, 2H, butyl), 1.39 (s, 9H, Boc), 1.44–1.70 (m, 4H, butyl+alkyl), 1.72–1.91 (m, 1H, alkyl), 1.99–2.12 (m, 1H, alkyl), 2.30–2.65 (m, 5H, butyl+alkyl), 4.35–4.68 (m, 2H, bridgeheads), 5.48–5.62 (m, 2H, olefinic); ^{13}C NMR δ 14.34 (3), 22.81 (2), 24.67, 25.38 (2), 26.41 (2), 28.64, 28.74 (3), 29.93, 30.94 (2), 32.55, 34.58 (2), 40.05, 40.59 (2), 55.15, 55.74 (1), 57.55, 58.04 (1), 61.19, 62.58 (1), 79.72, 80.50 (0), 125.94, 126.82 (1), 132.79, 134.00 (1), 153.77 (0), 210.08 (0); EIMS *m/z* 307 (M^+, 14), 251 ($M^+-(H_3C)_2CCH_2$, 57), 243 ($M^+-(H_3C)_2CCH-H_2O$, 37), 207 (M^+-Boc, 81); HRMS *m/z* calcd for $C_{18}H_{29}NO_3$ (M^+) 307.2147, found 307.2138.

2-Acetyl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-nonane (14a). A suspension of the alkene **13a** (4.8 g, 18 mmol) and 20% Pd/C (960 mg) in methanol (120 mL) was hydrogenated (balloon) for 3 h. The catalyst was filtered off and thoroughly washed with methanol (50 mL), and the combined filtrates were evaporated. The residue was diluted with ether (150 mL), washed with saturated $NaHCO_3$ (1×30 mL), brine (1×30 mL), and dried. Filtration and evaporation provided a pale oil which was purified by column chromatography (elution with 3:2 ether–petroleum ether) to yield **14a** as a colourless oil (4.6 g, 95%): TLC (ether–hexane) R_f 0.23; IR (neat) 1710, 1687 cm^{-1}; ^{1}H NMR δ (two rotamers 9:10) 1.43 and 1.45 (s, 9H, Boc), 1.47–2.30 (m, 10H, alkyl), 2.21 and 2.25 (s, 3H, methyl), 2.49–2.53 (m, 1H, alkyl), 4.15 and 4.32 (m, 1H, bridgehead), 4.45 and 4.55 (m, 1H, bridgehead); ^{13}C NMR δ 23.33, 23.53 (2), 27.80, 28.16 (2), 29.07, 29.24, 29.69 (3), 29.79, 29.93 (1), 34.37, 35.14, 35.43, 36.98 (2), 57.14, 57.63 (1), 62.40, 62.92 (1), 80.61, 81.51 (0), 154.62, 155.10 (0), 210.07, 211.31 (0); EIMS *m/z* (relative intensity) 267 (M^+, 13), 194 ($M^+-(H_3C)_2CCH_2-H_2O$, 24), 167 (M^+-Boc, 60), 149 (13), 124 (54), 96 (55), 82 (61), 57 (100); HRMS, *m/z* calcd for $C_{15}H_{25}NO_3$ (M^+) 267.1834, found 267.1829.

2-Benzoyl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-nonane (14b). Prepared according to the general procedure. Purification was carried out by flash column chromatography eluting with 2:3 ether–petroleum ether, to afford the title compound as a white solid (4.66 g, 95%): mp 133–135°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.44; IR ($CHCl_3$) 2932, 1684 cm^{-1}; ^{1}H NMR δ 1.26, 1.36 (s, 9H, Boc), 1.41–2.09 (m, 10H, alkyl), 2.38–2.51 (m, 1H, alkyl), 3.15–3.20 (m, 1H, alkyl), 4.07–4.45 (m, 2H, bridgeheads), 7.22–7.46 (m, 3H, aromatic), 7.69–7.80 (m, 2H, aromatic) ppm; ^{13}C NMR δ 22.45 (2), 26.66, 26.70, 26.83 (2), 28.64, 28.88 (3), 33.16 (2), 34.55 (2), 35.91 (2), 56.38, 56.61, 56.81 (1), 80.13 (0), 128.91, 129.02, 129.05 (1), 133.05 (1), 136.43 (0), 153.63 (0), 200.40 (0) ppm; EIMS *m/z* (relative intensity) 329 (M^+, 17), 273 ($M^+-(H_3C)_2CCH_2$, 14), 256 ($M^+-(H_3C)_2CCH-H_2O$, 33), 229 (M^+-Boc, 92); HRMS *m/z* calcd for $C_{20}H_{27}NO_3$ (M^+) 329.1991, found 329.1200.

2-Valeryl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]nonane (14c). Prepared according to the general procedure. Purification was carried out by flash column chromatography eluting with 3:1 petroleum ether–ether, to afford the title compound as a colourless oil (266 mg, 77%): TLC (1:3 ether–petroleum ether) R_f 0.16; IR (film) 2932, 1692 cm^{-1}; ^{1}H NMR δ 0.78–0.90 (m, 3H, butyl), 1.11–1.29 (m, 3H, butyl+alkyl), 1.33 and 1.38 (s, 9H, Boc), 1.40–1.65 (m, 5H, butyl+alkyl), 1.66–1.88 (m, 4H, alkyl), 1.88–2.09 (m, 1H, alkyl), 2.17–2.58 (m, 4H, butyl+alkyl), 4.09–4.52 (m, 2H, bridgeheads) ppm; ^{13}C NMR δ 14.36 (3), 22.52, 22.85 (2), 26.57, 26.62, 26.68, 26.85 (2), 28.00 (2), 28.64 (3), 28.85 (1), 32.87 (2), 33.90, 34.46 (2), 36.46 (2), 39.90, 40.84 (2), 56.33, 56.54 (1), 60.85, 61.43 (1), 79.42, 80.25 (0), 153.62 (0), 211.03, 212.10 (0) ppm; EIMS *m/z* (relative intensity) 309 (M^+, 18), 253 ($M^+-(H_3C)_2CCH_2$, 9), 236 ($M^+-(H_3C)_2CCH-H_2O$, 22), 209 (M^+-Boc, 57); HRMS *m/z* calcd for $C_{18}H_{31}NO_3$ (M^+) 309.2304, found 309.2305.

9-(*tert*-Butoxycarbonyl)-2-((*Z*)-1-(dimethyl-*tert*-butylsiloxy)ethylidene)-9-azabicyclo[4.2.1]nonane (15a). This was prepared exactly according to the method of Rapoport,[14] using **14a** (610 mg, 2.3 mmol) to yield **15a** as a colourless oil (820 mg, 94%): ^{1}H NMR δ (two rotamers) 0.04, 0.09, 0.10 and 0.13 (s, 6H, TBS), 0.79 and 0.80 (s, 9H, TBS), 1.1–2.1 (m, 9H, alkyl), 1.28 and 1.31 (s, 9H, Boc), 1.67 (s, 3H, methyl), 2.15 (dd, *J*=14.7, 7.0 Hz, 1H, alkyl),

4.11 and 4.23 (m, 1H, bridgehead), 4.72 and 4.76 (d, J=8.4 Hz, 1H, bridgehead).

9-(*tert*-Butoxycarbonyl)-2-((*Z*)-1-(dimethyl-*tert*-butylsiloxy)benzylidene)-9-azabicyclo[4.2.1]nonane (15b). This was prepared exactly according to the method of Rapoport,[14] using **14b** (485 mg, 1.47 mmol) to yield **15b** as a colourless oil (94%): TLC (1:9 ether–petroleum ether) R_f 0.29; IR ($CHCl_3$) 2928, 1695, 1653, 1252, 1106 cm^{-1}; ^1H NMR δ −0.19 (s, 3H, TBS), 0.00 (s, 2H, TBS), 0.05 (s, 1H, TBS), 0.95–1.09 (m, 9H, TBS), 1.32–1.82 (m, 13H, alkyl+Boc), 1.82–2.28 (m, 4H, alkyl), 2.32–2.58 (m, 2H, alkyl), 4.40–4.59 (m, 1H, bridgehead), 5.13–5.24 (m, 1H, bridgehead), 7.32–7.50 (m, 5H, aromatic) ppm; ^{13}C NMR δ 18.50 (0), 24.48, 24.65 (2), 26.09, 26.17, 26.23 (3), 26.50, 26.65 (2), 28.54 (3), 28.93 (2), 29.03, 29.08, 29.18 (3), 32.79, 33.45, 33.74, 34.15 (2), 56.81, 56.97 (1), 78.92, 79.23 (0), 125.84, 126.23 (0), 127.89, 128.00, 128.16, 128.27 (1), 130.03, 130.16, 130.28 (1), 139.14, 139.27 (0), 143.79, 144.30 (0), 154.06 (0) ppm; EIMS m/z (relative intensity) 443 (M^+, 30), 386 (M^+−*tert*-Bu, 18), 370 ($M^+-(H_3C)_2CCH-H_2O$, 14), 342 (M^+−Boc, 32); HRMS m/z calcd for $C_{26}H_{41}NO_3Si$ (M^+) 443.2856, found 443.2840.

9-(*tert*-Butoxycarbonyl)-2-((*Z*)-1-(dimethyl-*tert*-butylsiloxy)pentylidene)-9-azabicyclo[4.2.1]nonane (15c). This was prepared exactly according to the method of Rapoport,[14] using **14c** (266 mg, 0.9 mmol) to yield **15c** as a colourless oil (65%): TLC (3:2 petroleum ether–ether) R_f 0.60; IR (film) 2929, 1699, 1657, 1258, 1109 cm^{-1}; ^1H-NMR δ −0.50 to 0.09 (m, 6H, TBS), 0.70–0.88 (m, 12H, TBS+butyl), 1.06–1.22 (m, 6H, butyl+alkyl), 1.27 and 1.30 (s, 9H, Boc), 1.35–1.70 (m, 5H, alkyl), 1.70–2.25 (m, 5H, butyl+alkyl), 4.04–4.27 (m, 1H, bridgehead), 4.67–4.77 (m, 1H, bridgehead) ppm; ^{13}C NMR δ 14.55 (3), 18.60 (0), 22.66 (2), 23.08 (2), 24.60 (2), 26.27 (3), 28.46 (2), 28.96 (3), 30.72 (2), 32.01 (2), 32.62 (2), 34.26 (2), 56.55 (1), 56.79 (1), 79.06 (0), 123.85 (0), 144.41 (0), 153.96 (0) ppm; EIMS m/z (relative intensity) 423 (M^+, 14), 350 ($M^+-(H_3C)_2CCH-H_2O$, 10), 322 (M^+−Boc, 25); HRMS calcd for $C_{24}H_{45}NO_3Si$ (M^+) 423.3169, found 423.3164.

2-Acetyl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-non-2-ene (16a). This was prepared exactly according to the method of Rapoport,[14] using **15a** (820 mg, 2.2 mmol) to yield **16a** as a colourless oil (460 mg, 81%): ^1H NMR δ (two rotamers) 1.38 and 1.45 (s, 9H), 1.6–1.7 (m, 3H), 2.1–2.5 (m, 5H), 2.30 (s, 3H), 4.30 and 4.42 (m, 1H), 5.14 and 5.19 (m, 1H), 6.84 (t, J=5.9 Hz, 1H). Boc-anatoxin was prepared from an authentic sample of anatoxin and gave identical TLC and ^1H NMR spectrum. Boc deprotection of (**16a**) was carried out using TFA to provide anatoxin, which gave identical TLC and ^1H NMR characteristics to the authentic sample.

2-Benzoyl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-non-2-ene (16b). This was prepared exactly according to the method of Rapoport,[14] using **15b** (450 mg, 1.02 mmol) to yield **16b** as a white solid (76%): mp 142–144°C (ether–petroleum ether); TLC (2:3 ether–petroleum ether) R_f 0.35; IR ($CHCl_3$) 2925, 1686, 1644 cm^{-1}; ^1H NMR δ 1.27, 1.41 (s, 9H, Boc), 1.56–1.88 (m, 3H, alkyl), 2.01–2.21 (m, 2H, alkyl), 2.30–2.51 (m, 3H, alkyl), 4.24–4.30, 4.39–4.47 (m, 1H, bridgehead), 4.96–5.10 (m, 1H, bridgehead), 6.24–6.40 (m, 1H, olefinic), 7.27–7.65 (m, 5H, aromatic) ppm; ^{13}C NMR δ 24.77 (2), 28.76 (3), 30.67 (2), 32.40 (2), 55.15 (1), 56.53 (1), 80.00 (0), 128.50 (1), 129.96 (1), 132.30 (1), 138.13 (0), 144.59 (1), 150.35 (0), 153.45 (0), 197.68 (0) ppm; EIMS m/z (relative intensity) 327 (M^+, 10), 271 ($M^+-(H_3C)_2CCH_2$, 18), 254 ($M^+-(H_3C)_2CCH-H_2O$, 9), 227 (M^+−Boc, 26); HRMS m/z calcd $C_{20}H_{25}NO_3$ (M^+) 327.1834, found 327.1835.

Deprotection was effected using the method reported by Gallagher et al.[23] The *N*-Boc derivative (**16b**) was dissolved in 1,4-dioxane, followed by the addition of 2 M hydrochloric acid. The reaction mixture was warmed to 50°C for 2 h, and then concentrated in vacuo to give an orange solid (100%): IR (CH_2Cl_2) 3401, 2924, 1642, 1596 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.77–1.85 (m, 1H, alkyl), 1.88–2.07 (m, 3H, alkyl), 2.09–2.19 (m, 1H, alkyl), 2.34–2.53 (m, 2H, alkyl), 2.55–2.65 (m, 1H, alkyl), 4.17–4.27 (m, 1H, bridgehead), 4.74–4.84 (m, 1H, bridgehead), 6.70–6.77 (m, 1H, olefinic), 7.46–7.73 (m, 5H, aromatic), 9.05–9.20 (m, 1H, N*H*), 9.69 (br s, 1H, N*H*) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 23.35 (2), 26.86 (2), 27.21 (2), 30.23 (2), 53.67 (1), 58.19 (1), 128.43 (1), 129.52 (1), 132.35 (1), 137.02 (0), 142.26 (0), 149.81 (1), 195.74 (0) ppm; EIMS m/z (relative intensity) 227 (M^+, 100), 198 (50), 184 (24), 170 (17), 122 (43); HRMS m/z calcd for $C_{15}H_{17}NO$ (M^+) 227.1310, found 227.1308.

2-Valeryl-9-(*tert*-butoxycarbonyl)-9-azabicyclo[4.2.1]-non-2-ene (16c). This was prepared exactly according to the method of Rapoport,[14] using **15c** (210 mg, 0.5 mmol) to yield **16c** as a colourless oil (73%): TLC (3:2 Petrol/Et_2O) R_f 0.41; IR ($CHCl_3$) 2960, 2932, 1694, 1632 cm^{-1}; ^1H NMR δ 0.91 (t, J=7.0 Hz, 3H, butyl), 1.13–1.40 (m, 13H, Boc+butyl+alkyl), 1.90–2.14 (m, 2H, alkyl), 2.29–2.41 (m, 2H, alkyl), 2.45–2.60 (m, 2H, butyl), 4.10–4.38 (m, 1H), 4.94–5.10 (m, 1H), 6.60–6.77 (m, 1H) ppm; ^{13}C NMR δ 14.35 (3), 22.93 (2), 24.56 (2), 27.58 (2), 28.85 (3), 30.12 (2), 30.87 (2), 32.02 (2), 37.35 (2), 53.64 (1), 56.00 (1), 79.69 (0), 141.26 (1), 150.27 (0), 153.55 (0), 200.73 (0) ppm; EIMS m/z (relative intensity) 307 (M^+, 21), 251 ($M^+-(CH_3)_2CCH_2$, 34), 234 ($M^+-(CH_3)_2CCH-H_2O$, 36), 207 (M^+−Boc, 69); HRMS m/z calcd for $C_{18}H_{29}NO_3$ (M^+) 307.2147, found 307.2143.

Deprotection was carried out using a modification to the method reported by Gallagher et al.[23] The *N*-Boc derivative (**16c**) was dissolved in 1,4-dioxane, followed by the addition of 2 M hydrochloric acid, and the reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo to afford a yellow oil (100%): IR ($CHCl_3$) 3407, 2959, 1662 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J=7.3 Hz, 3H, butyl), 1.22–1.32 (m, 2H, butyl), 1.47 (pent, J=7.3 Hz, 2H, butyl), 1.80 (br s, 3H, alkyl), 1.96 (br t, J=10.1 Hz, 1H, alkyl), 2.06–2.09 (m, 1H, alkyl), 2.25–2.30 (m, 2H, alkyl), 2.58–2.63 (m, 1H, alkyl), 2.65–2.69 (m, 2H, butyl), 4.13 (br s, 1H, bridgehead), 4.84 (br s, 1H, bridgehead), 7.33–7.34 (m, 1H, olefinic), 8.71 (br s, 1H, N*H*), 9.68 (br s, 1H, N*H*) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6) 13.67 (3), 21.63 (2), 22.92 (2), 26.23 (2), 26.71

(2), 27.14 (2), 29.69 (2), 35.71 (2), 51.38 (1), 57.69 (1), 142.50 (0), 146.46 (1), 198. 76 (1) ppm; EIMS *m/z* (relative intensity) 242 (6, $(M-H)^+HCl$), 206 (69, $(M-H)^+$), 178 (17), 137 (16), 122 (25); HRMS *m/z* calcd for $C_{13}H_{20}NO$ ($(M-H)^+$) 206.1545, found 206.1530.

Acknowledgements

We thank Professor R.C. Cookson for his continued interest in this work and Tocris-Cookson Ltd for generous financial support. We thank Richard Parlitz for technical assistance and Dr Tony Avent for obtaining NMR spectra at 500 MHz.

References

1. Huber, C. S. *Acta Crystallogr., Sect. B* **1972**, *78*, 2577.
2. Devlin, J. P.; Edwards, O. E.; Gorham, P. R.; Hunter, N. R.; Pike, R. K.; Stavric, B. *Can. J. Chem.* **1977**, *55*, 1367.
3. Mansell, H. L. *Tetrahedron* **1996**, *52*, 6025 and references cited therein.
4. Carmichael, W. W.; Biggs, D. F.; Gorham, P. R. *Science* **1975**, *187*, 542.
5. Spivak, C. E.; Witkop, B.; Alburquerque, E. X. *Mol. Pharmacol.* **1980**, *18*, 384.
6. Koskinen, A. M. P.; Rapoport, H. *J. Med. Chem.* **1985**, *28*, 1301.
7. Swanson, K. L.; Aronstam, R. S.; Wonnacott, S.; Rapoport, H.; Albuquerque, E. X. *J. Pharmacol. Exp. Ther.* **1991**, *259*, 377.
8. Wonnacott, S.; Jackman, S.; Swanson, K. L.; Rapoport, H.; Albuquerque, E. X. *J. Pharmacol. Exp. Ther.* **1991**, *259*, 387.
9. Magnus, N. A.; Ducry, L.; Rolland, V.; Wonnacott, S.; Gallagher, T. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2313.
10. Wright, E.; Gallagher, T.; Sharples, C. G. V.; Wonnacott, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2867.
11. Oh, C. Y.; Kim, K. S.; Ham, W. H. *Tetrahedron Lett.* **1998**, *39*, 2133.
12. Parsons, P. J.; Camp, N. P.; Underwood, J. M.; Harvey, D. M. *J. Chem. Soc., Chem. Commun.* **1995**, 1461.
13. Parsons, P. J.; Camp, N. P.; Underwood, J. M.; Harvey, D. M. *Tetrahedron* **1996**, *52*, 11637.
14. Sardina, F. J.; Howard, M. H.; Koskinen, A. M. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 4654.
15. Baldwin, J. E.; Adlington, R. M.; Gollins, D. W.; Schofield, C. J. *Tetrahedron* **1990**, *46*, 4733.
16. Ohta, T.; Hosoi, A.; Kimura, T.; Nozoe, S. *Chem. Lett.* **1987**, 2091.
17. Clive, D. L. J.; Wong, C. K.; Kiel, W. A.; Menchen, S. M. *J. Chem. Soc., Chem. Commun.* **1978**, 379.
18. Nicolaou, K. C.; Claremon, D. A.; Barnette, W. E.; Seitz, S. P. *J. Am. Chem. Soc.* **1979**, *101*, 3704.
19. Clive, D. L. J.; Farina, V.; Singh, A.; Wong, C. K.; Kiel, W. A.; Menchen, S. M. *J. Org. Chem.* **1980**, *45*, 2120.
20. Toshmitsu, A.; Terao, K.; Vemura, S. *Tetrahedron Lett.* **1984**, *25*, 5917.
21. Grieco, P.; Nishizawa, M.; Marisovic, N. *J. Am. Chem. Soc.* **1976**, *98*, 7102.
22. Sardina, F. J.; Howard, M. H.; Morningstar, M.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 5025.
23. Thomas, P.; Brough, P. A.; Gallagher, T.; Wonnacott, S. *Drug Dev. Res.* **1994**, *31*, 147.