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Reactive intermediates in peptide synthesis. *ortho*-Nitrophenyl N^{α}-*para*-toluenesulfonyl-α-aminoisobutyrate

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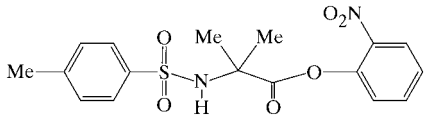
The preparation, characterization, and molecular and crystal structures of the title compound [IUPAC name: 2-nitrophenyl 2-methyl-2-(*para*-toluenesulfonylamino)propanoate], $C_{17}H_{18}N_2O_6S$, are reported. The phenyl group is almost perpendicular to the plane of the adjacent ester moiety. One O atom of the nitro group is wedged between the two ester O atoms. The implications of this peculiar conformation for the chemistry of *ortho*-nitrophenyl esters in peptide synthesis are discussed.

Comment

In a recent review article (Toniolo *et al.*, 1996), we have shown that X-ray diffraction may provide a body of valuable information on the geometry and conformation of reactive amino acid derivatives in peptide synthesis. These parameters, in turn, are extremely useful for the understanding of amino acid reactivity, regiospecificity and racemization tendency. We also anticipated that a more systematic exploitation of this technique, in combination with theoretical studies and kinetic experiments, would allow a complete definition of the mechanisms operative in peptide bond formation and in undesired side reactions. By using a high-molecular-weight aromatic N^{α}-protecting group, such as Tos (*para*-toluenesulfonyl), and a conformationally restricted α-amino acid, such as the $C^{\alpha,\alpha}$-disubstituted glycine Aib (α-amino-isobutyric acid), we were able to overcome the drawbacks of low melting points and poor crystallinities generally suffered by ephemeral reactive intermediates in peptide synthesis.

In this communication, we describe the synthesis and characterization, and the results of a crystallographic analysis of the title compound, Tos-Aib-ONP$_o$, (I) (NP$_o$ is *ortho*-nitrophenyl). The nitrophenyl esters of N^{α}-protected α-amino acids were introduced in peptide synthesis almost 50 years ago (Bodanszky, 1955). The enhancement of the electron-withdrawing properties of the aromatic nucleus by the strong effect of a nitro group makes these active esters useful intermediates that have been applied in the synthesis of many important peptides. Their general exploitation was first demonstrated in the synthesis of oxytocin (Bodanszky & Du Vigneaud, 1959).



(I)

The higher aminolysis rates subsequently noted in the *ortho*-nitrophenyl esters of N^{α}-urethane-protected α-amino acids compared with the *para* isomers could readily be explained by the electron-withdrawing effects of the nitro group being transferred to the ester carbonyl not merely by resonance, as in the *para*-nitrophenyl derivatives, but also through a σ bond (Bodanszky & Bath, 1969). It was less easy to find a satisfactory explanation for the lower sensitivity of the *ortho* isomers with respect to solvent effects and steric hindrance. An in-depth examination (Bodanszky *et al.*, 1974) of a number of *ortho*- and *para*-nitrophenyl esters revealed that, in the *ortho* series, the values of specific rotation are consistently higher and the NMR spectra indicate greater anisotropy. All these experimental data point to restricted rotation around bonds near the chiral centre. It became obvious that some kind of intramolecular interaction of the nitro group ought to be responsible for the unusually rigid geometry in the molecules of the *ortho* isomers. Since no intramolecular hydrogen bond could be found, a dipole–dipole interaction between the nitro group and the N^{α}-protecting urethane grouping was invoked as the stabilizing force. The intramolecular involvement of the nitro group should lead to a decrease in the influence of solvents on reaction rates, because, in all probability, the nitro group is the moiety most responsible for the interaction of nitrophenyl esters with solvent molecules. Also, since less solvated molecules can better penetrate the crowded environment of insoluble polymeric supports, the observation that *ortho*-nitrophenyl esters are more efficient in solid-phase synthesis than their *para*-substituted analogues found a reasonable explanation. Thus, the postulated intramolecular involvement of the nitro group in *ortho*-nitrophenyl esters appeared to be the common factor responsible for all observed phenomena. Therefore, it was desirable to seek supporting evidence for the correctness of this assumption by means of X-ray crystallography.

In (I), the *ortho*-nitrophenyl group exhibits a remarkable conformation with respect to the remainder of the molecule (Fig. 1). The phenyl group is almost perpendicular [77.9 (2)°] to the plane of the adjacent ester moiety. One O atom of the nitro group (O5) is wedged between the two ester O atoms (O3 and O4), so that the possible rotation about the C12—O4 bond is restricted. The nitro group is rotated about the N2—C17 bond by 11° from the plane of the phenyl group. Short intramolecular distances involve atom O5 of the nitro group

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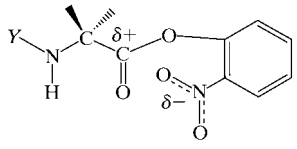
and atoms O3 and O4 of the –C(O)O– ester grouping [O5···O3 3.064 (4) Å and O5···O4 2.660 (4) Å]. The ester group is planar, with the largest deviation being 0.013 Å for C11.

The C11═O3 bond length of 1.199 (3) Å (Table 1) is typical of carboxylic esters (1.196 Å; Allen *et al.*, 1987). The sum of the bond angles around the carbonyl atom C11 is 359.9°, an indication of the planarity of the carboxylic ester moiety. The C12—O4 bond of 1.399 (3) Å is significantly shorter than the average value found in alkyl esters (Schweizer & Dunitz, 1982), as a consequence of the conjugation of the nitro-aromatic π system with the lone-pair electrons of the ester O atom.

The crystal state conformation of (I) vindicates the assumption of an intramolecular involvement of the nitro group, although the latter interacts with the ester carbonyl, rather than with the N^{α}-protecting group as proposed earlier (Bodanszky *et al.*, 1974). It seems appropriate to attribute this proximity, and the consequent rigid bending of the ester moiety, to coulombic attraction between the partially positively charged carbonyl C atom and the partially negatively charged O atom of the nitro group; the *Scheme* below shows this proposed intramolecular electrostatic attraction in an *ortho*-nitrophenyl ester of an N^{α}-protected α-amino acid. This



explanation is based on the generally accepted electron distribution in active esters. No similar interaction is possible in the *para*-nitrophenyl esters. The unique geometry of the –ONP$_o$ ester in the molecule of (I) provides a satisfactory explanation for its special properties. Because of the bulky rigidly bent ester, and the nearby rotation around the bond connecting the C8 atom with the carbonyl atom C11 being considerably restricted, fewer rotamers can exist in an –ONP$_o$ ester than in an –ONP$_p$ ester and, accordingly, a higher optical rotation is associated with the former compound.

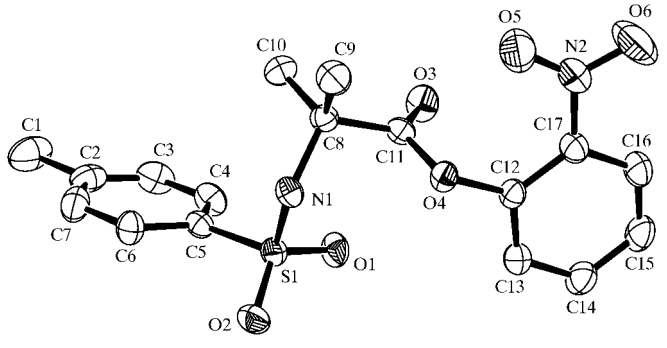


Figure 1
A view of the molecule of (I) with the atom-numbering scheme. Displacement ellipsoids are shown at the 30% probability level and H atoms have been omitted for clarity.

The intramolecular involvement of the nitro group limits interactions with solvent molecules and consequently reduces the effect of solvent on reactivity. Also, these molecules, with their smaller solvated shell, can readily diffuse into a polymer matrix and perform better in solid-phase synthesis than their *para*-substituted analogues. It should also be noted that, in spite of the crowding that follows from the proximity of the nitro and carbonyl groups, one side of the latter is not encumbered and, consequently, it is exposed to the attack of the incoming nucleophile.

The geometry and conformation of another crystalline –ONP$_o$ ester, Boc-L-Ala-ONP$_o$ (Boc is *tert*-butyloxycarbonyl) are similar. In particular, the nitro group is rotated by 22° with respect to the adjacent phenyl ring (Karle & Bodanszky, 1988).

The φ (S1—N1—C8—C11) and ψ (N1—C8—C11—O4) amino acid backbone torsion angles (IUPAC–IUB Commission on Biochemical Nomenclature, 1970) are −61.5 (3) and −52.5 (3)°, respectively. This pair of angles confirms the tendency of the Aib residue to fold into a helical structure (Karle & Balaram, 1990; Toniolo & Benedetti, 1991). The conformationally sensitive Aib τ (N1—C8—C11) bond angle (Paterson *et al.*, 1981) of 108.4 (2)° is close to the tetrahedral value. The C5—S1—N1—C8 torsion angle is −75.9 (2)°.

Significantly short intramolecular distances between non-bonded atoms in (I) are O1···C11 2.795 (3) Å and O1···O3 3.204 (4) Å. The S1 atom is 2.755 (3) Å from atom C8, 3.335 (3) Å from C10 and 3.3228 (3) Å from C11. The dihedral angle between normals to the average planes of the aromatic ring of the Tos group and the ester –C(O)O– group is 162.2 (2)°, while that involving the Tos and nitrophenyl aromatic rings is 85.6 (1)°.

In the crystal structure of (I), the molecules are linked through intermolecular N1—H1···O3═C11(x, $-y$, $z + \frac{1}{2}$) hydrogen bonds, giving rise to rows of molecules along the c direction. The N1···O3 distance of 2.894 (3) Å, and the N1—H1···O3 angle of 131° (Table 2), are in the usual range (Görbitz, 1989).

Experimental

For the synthesis of (I), Tos-Aib-OH (257 mg, 1 mmol; Leplawy *et al.*, 1960) and *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride (192 mg, 1 mmol) were dissolved at 273 K in 5 ml of anhydrous pyridine in the presence of *ortho*-nitrophenol (208 mg, 1.5 mmol). The reaction mixture was stirred for 30 min at 273 K and then for 4 h at room temperature. The solvent was evaporated, and the residue was taken up in ethyl acetate and washed with 0.5 *M* citric acid, water, 5% $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Compound (I) was obtained in 72% yield from ethyl acetate–petroleum ether (1:4) as a colourless solid with a melting point of 413–414 K. Single crystals of (I) were obtained from an ethanol solution by slow evaporation. Thin-layer chromatography, R_F ($CHCl_3$–ethanol, 9:1): 0.85; IR (KBr, cm^{-1}): 3276, 1773; ^{1}H NMR (200 MHz, $CDCl_3$, δ, p.p.m.): 8.12–7.30 (8H, 8 aromatic CH), 5.28 (1H, Aib NH), 2.43 (3H, Tos CH_3), 1.58 (6H, 2 Aib CH_3).

Crystal data

$C_{17}H_{18}N_2O_6S$
M_r = 378.39
Monoclinic, $C2/c$
a = 36.449 (3) Å
b = 9.082 (2) Å
c = 11.636 (2) Å
β = 106.10 (7)°
V = 3700.8 (17) Å^3
Z = 8
D_x = 1.358 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 48 reflections
θ = 7–12°
μ = 0.21 mm^{-1}
T = 293 (2) K
Prism, colourless
0.5 × 0.3 × 0.3 mm

Data collection

Philips PW1100 diffractometer
$\theta/2\theta$ scans
3981 measured reflections
3785 independent reflections
2144 reflections with $I > 2\sigma(I)$
R_{int} = 0.021
θ_{max} = 26.4°
$h = -45 \rightarrow 43$
$k = 0 \rightarrow 11$
$l = 0 \rightarrow 14$
3 standard reflections
every 50 reflections
intensity decay: negligible

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.047
$wR(F^2)$ = 0.144
S = 0.92
3785 reflections
237 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0825P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.32 e Å^{-3}
$\Delta\rho_{min}$ = −0.33 e Å^{-3}

Table 1
Selected geometric parameters (Å, °).

S1—N1	1.614 (2)	O6—N2	1.210 (4)
O4—C11	1.348 (3)	N1—C8	1.485 (3)
O4—C12	1.399 (3)	N2—C17	1.463 (4)
O3—C11	1.199 (3)	C8—C11	1.521 (3)
O5—N2	1.186 (3)	C12—C17	1.390 (4)
C11—O4—C12	116.22 (18)	O3—C11—C8	125.0 (2)
O5—N2—O6	120.7 (3)	O4—C11—C8	113.00 (19)
O5—N2—C17	120.7 (3)	C13—C12—C17	119.9 (2)
O6—N2—C17	118.4 (3)	C15—C16—C17	120.5 (3)
N1—C8—C11	108.4 (2)	C12—C17—C16	119.2 (3)
O3—C11—O4	121.9 (2)		

The methyl H atoms on atom C1 were modelled on two sets of positions, rotated from each other by 60° and with occupancy factors of 0.5, while the remaining H atoms were calculated at idealized positions. All H atoms were refined as riding, with N—H = 0.86 Å and C—H = 0.93–0.96 Å, and with U_{iso}(H) = 1.2 (or 1.5 for the methyl groups) times the U_{eq} of the parent atom.

Table 2
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
N1—H1···O3^{i}	0.86	2.26	2.894 (3)	131

Symmetry code: (i) $x, -y, \frac{1}{2}+z$.

Data collection: *FEBO* (Belletti, 1993); cell refinement: *FEBO*; data reduction: *FEBO*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*-3 *for Windows* (Farrugia, 1997); software used to prepare material for publication: *PARST*96 (Nardelli, 1995).

Supplementary data for this paper are available from the IUCr electronic archives (Reference: SK1532). Services for accessing these data are described at the back of the journal.

References

Allen, F. H., Kennard, O., Watson, D. G., Brammer, L., Orpen, A. G. & Taylor, R. (1987). *J. Chem. Soc. Perkin Trans.* 2, pp. S1–19.
Belletti, D. (1993). *FEBO*. Internal Report 1/93. Centro di Studio per la Strutturistica Diffrattometrica del CNR, Parma, Italy.
Bodanszky, M. (1955). *Nature* (*London*), **175**, 685–686.
Bodanszky, M. & Bath, R. J. (1969). *J. Chem. Soc. Chem. Commun.* pp. 1259–1260.
Bodanszky, M. & Du Vigneaud, V. (1959). *Nature* (*London*), **183**, 1324–1325.
Bodanszky, M., Fink, M. L., Funk, K. W., Kondo, M., Yang Lin, C. & Bodanszky, A. (1974). *J. Am. Chem. Soc.* **96**, 2234–2240.
Farrugia, L. J. (1997). *J. Appl. Cryst.* **30**, 565.
Görbitz, C. H. (1989). *Acta Cryst.* B**45**, 390–395.
IUPAC-IUB Commission on Biochemical Nomenclature (1970). *Biochemistry*, **9**, 3471–3479.
Karle, I. L. & Balaram, P. (1990). *Biochemistry*, **29**, 6747–6756.
Karle, I. L. & Bodanszky, M. (1988). *Int. J. Pept. Protein Res.* **32**, 194–199.
Leplawy, M. T., Jones, D. S., Kenner, G. W. & Sheppard, R. C. (1960). *Tetrahedron*, **11**, 39–51.
Nardelli, M. (1995). *J. Appl. Cryst.* **28**, 659.
Paterson, Y., Rumsey, S. M., Benedetti, E., Némethy, G. & Scheraga, H. A. (1981). *J. Am. Chem. Soc.* **103**, 2947–2955.
Schweizer, W. B. & Dunitz, J. D. (1982). *Helv. Chim. Acta*, **65**, 1547–1554.
Sheldrick, G. M. (1997). *SHELXS*97 and *SHELXL*97. University of Göttingen, Germany.
Toniolo, C. & Benedetti, E. (1991). *Macromolecules*, **24**, 4004–4009.
Toniolo, C., Crisma, M. & Formaggio, F. (1996). *Biopolymers*, **40**, 627–651.