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Glycine-selective α-Carbon-Nitrogen Bond Cleavage of Dipeptides by Nickel Peroxide

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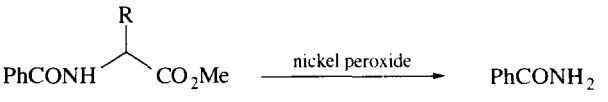
Abstract: Nickel peroxide selectively cleaves the α-carbon-nitrogen bond of glycine residues in dipeptide derivatives to give the corresponding amides. The glycine selectivity is attributable to preferential complexation of the reactant residue to nickel peroxide and subsequent reaction *via* a stable α-centred glycyl radical. The oxidation process serves as a chemical model for peptidylglycine α-amidating monooxygenase (PAM) and, in addition, may have potential for the synthesis of α,β-didehydro amino acid residues within peptides. © 1997 Elsevier Science Ltd.

Nickel peroxide, obtained by the action of alkaline hypochlorite on a nickel (II) salt,[1] is a black, high valency, non-stoichiometric oxide of nickel which is useful as an oxidant of a variety of organic substrates in both aqueous and organic solvents.[2] The free radical nature of oxidations with nickel peroxide has been established in deuterium isotope experiments and electron spin resonance (esr) studies with radical spin traps,[3,4] and the mechanism of reaction is in general considered to involve both hydrogen atom abstraction and hydroxyl radical donation by nickel peroxide.[2,5]

We have investigated nickel peroxide oxidation of amino acid derivatives as a chemical model for peptidylglycine α-amidating monooxygenase (PAM). In the preliminary report[6] of our work in this area it was demonstrated that the *N*-benzoyl amino acid derivatives **1a** – **c** reacted by oxidative cleavage of the α-carbon-nitrogen bond to give benzamide (**2**) in each case (*Scheme 1*). The reactions were selective for cleavage of the glycine derivative **1a**, such that in competitive experiments using mixtures of the alanine derivative **1b** with either the glycine derivative **1a** or the valine derivative **1c**, each at 0.025 mol dm^{-3} in benzene at 80 °C, the glycine derivative **1a** reacted with nickel peroxide 10.4 ± 2.5 times faster than the alanine derivative **1b**, which in turn reacted 7.0 ± 1.5 times faster than the valine derivative **1c**.



1 **2**

a: R = H
b: R = Me
c: R = $CHMe_2$

Scheme 1

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In probing the basis of the glycine selectivity, we have now examined reactions of the dipeptide derivatives **3a – c** and **5a – d** with nickel peroxide. In the particular cases of the dipeptide derivatives **5c** and **5d**, reaction with nickel peroxide provides convenient access to α,β-didehydro amino acid derivatives. Reactions of the deuterated glycine derivative **11** and the sarcosine derivative **12** have also been investigated, and the outcome of these reactions, along with the reactions of the dipeptide derivatives **3a – c** and **5a – d** has aided elucidation of the reaction mechanism.

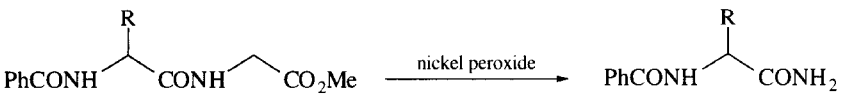
RESULTS AND DISCUSSION

The glycine-containing dipeptide derivatives **3a – c** and **5a,b** reacted upon treatment with nickel peroxide in refluxing benzene to give the corresponding amides **4a – c** and **8a,b** (*Table 1*). No amide bond cleavage was observed in these reactions and the product amides **4a – c** and **8a,b** arise as a result of oxidative cleavage of the α-carbon-nitrogen bond of the *C*-terminal glycine residue in each case (*Schemes 2 and 3*). The aspartic acid containing dipeptide derivatives **5c** and **5d**, however, reacted with nickel peroxide to afford the didehydroaspartate derivatives **10c** and **10d**, as well as the amide **8c**, in the case of **5c** (*Table 1*). The assignment of *Z*-stereochemistry to the dehydropeptides **10c** and **10d** was made on the basis of the tendency of dehydro amino acid derivatives to favour this configuration.[7]

Table 1. Reactions of the dipeptide derivatives **3a – c** and **5a – d** with nickel peroxide.

Substrate	Product	Yield†	Corrected Yield††
3a	**4a**	23%	74%
3b	**4b**	27%	68%
3c	**4c**	37%	46%
5a	**8a**	54%	79%
5b	**8b**	41%	55%
5c	**8c**	21%	33%
	10c	17%	27%
5d	**10d**	54%	86%

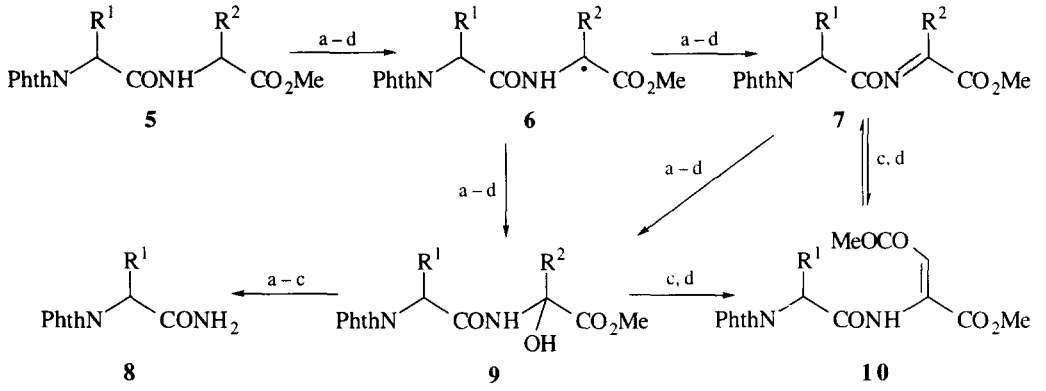
† yields not optimised †† based on recovered starting material



3 **4**

a: R = Me
b: R = $CHMe_2$
c: R = CH_2CHMe_2

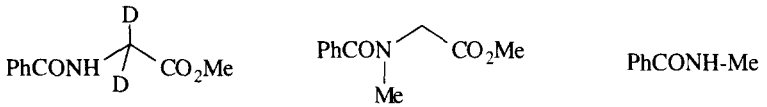
Scheme 2



a: $R^1 = CHMe_2$, $R^2 = H$
b: $R^1 = CH_2CHMe_2$, $R^2 = H$
c: $R^1 = CH_2Ph$, $R^2 = CH_2CO_2Me$
d: $R^1 = H$, $R^2 = CH_2CO_2Me$

Scheme 3

Production of the amides **4a – c** and **8a – c** from the glycyl dipeptides **3a – c** and **5a – c**, and of the didehydroaspartate derivatives **10c** and **10d** from the aspartyl dipeptides **5c** and **5d** may be rationalised as outlined in *Scheme 3*, for the case of the dipeptides **5a – d**. Following complexation to nickel, hydrogen atom transfer from the substrates **5a – d** affords the corresponding α-carbon-centred radicals **6a – d**, which combine with hydroxyl radical from nickel peroxide to give the corresponding α-hydroxy amino acid derivatives **9a – d**. Alternatively, the α-centred radicals **6a – d** may form the corresponding *N*-acylimines **7a – d**, *via* a second hydrogen atom transfer, followed by addition of water to give the corresponding α-hydroxy amino acid derivatives **9a – d**. Subsequent hydrolysis of the α-hydroxy amino acid derivatives **9a – c** then affords the respective amides **8a – c**. Formation of the dehydroaspartate derivatives **10c** and **10d** from the dipeptides **5c** and **5d** is attributable to either the elimination of water from the alcohols **9c** and **9d** or tautomerisation of the intermediate *N*-acylimines **7c** and **7d** (*Scheme 3*). This process is presumably favoured for aspartate residues due to extended conjugation in the products.



11 **12** **13**

Supporting evidence for the mechanism described above is provided by reactions of nickel peroxide with the dideuteroglycine derivative **11** and the *N*-benzoylsarcosine derivative **12**. The deuterated glycine derivative **11** reacted upon treatment with nickel peroxide to give benzamide (**2**) and recovered starting material **11**, for which the isotopic ratio was little changed. This indicates that the deuterium label is not exchanged under the reaction conditions and further, that the reaction with nickel peroxide is irreversible. In a competitive experiment using an equimolar mixture of substrates, the glycine derivative **1a** reacted 2.9 ± 0.5 times faster than its deuterated analogue **11**, representing a deuterium isotope effect consistent with that reported for

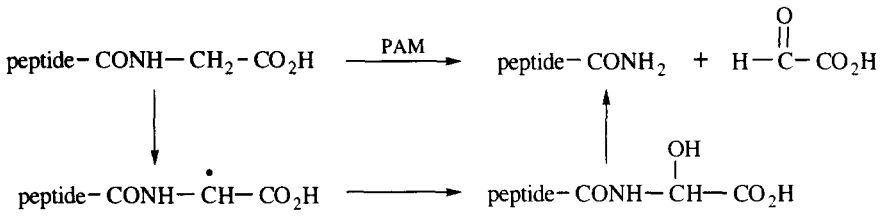
α-hydrogen atom transfer from amino acid derivatives under free radical conditions.[8] This, in turn, indicates that α-carbon-hydrogen bond homolysis is an irreversible rate-determining step in reactions of the dipeptide derivatives **3a – c** and **5a – d** with nickel peroxide.

The *N*-benzoylsarcosine derivative **12** reacted with nickel peroxide to give *N*-methylbenzamide (**13**) and benzamide (**2**). In a separate experiment, the nickel peroxide oxidation of *N*-methylbenzamide (**13**) gave benzamide (**2**), consistent with the amide **13** being the initial oxidation product of the sarcosine derivative **12**. As imine formation is not possible in the oxidation of the sarcosine derivative **12**, this establishes that oxidative α-carbon-nitrogen bond cleavage of amino acid derivatives by nickel peroxide can occur *via* direct hydroxylation of intermediate α-centred amino acid radicals, and need not involve imine intermediates.

Selective reaction at the *C*-terminal residues in the dipetides **5c** and **5d** is presumably due to the deactivating effect of the *N*-phthaloyl substituent[9] toward hydrogen atom abstraction at the adjacent carbon. When a single diastereomer of the dipeptide **5c** was treated with nickel peroxide, both **10c** and **8c** were produced without racemisation of the *N*-terminal phenylalanine residue. This example serves to illustrate the utility of the nickel peroxide oxidation procedure as methodology for *in situ* synthesis of α,β-didehydroamino acid residues within peptides.

The reactions of the dipeptide derivatives **3a – c** and **5a,b** to give the respective amides **4a – c** and **8a,b** each demonstrate selective cleavage by nickel peroxide of glycine residues. Whereas reaction at the *C*-terminal glycine residue in each of the phthaloyl substituted dipeptide derivatives **5a** and **5b** may simply reflect the deactivating effect of the *N*-phthaloyl substituent toward hydrogen atom abstraction from the adjacent carbon,[9] cleavage of the *C*-terminal residue in the reaction of each of the benzoyl substituted dipeptide derivatives **3a – c** clearly demonstrates a selectivity for reaction of glycine residues, as radical reactions of dipeptide derivatives of this type are normally selective for reaction of the *N*-terminal amino acid residue.[10] In contrast to reaction of the amino acid derivatives **1a – c**, wherein the selectivity for reaction of the glycine derivative **1a** may be affected by the relative solubilities of the reactant substrates in the reaction medium, insofar as this affects their relative ease of complexation to nickel peroxide,[6] the selectivity for reaction of the glycine residues in the dipeptides **3a – c** and **5a,b** is not affected by the individual solubilities of the dipeptides. Consequently, the selectivity for reaction of the glycine residues in the dipeptides **3a – c** and **5a,b** must be attributed to preferential complexation of the reactant residue to the nickel peroxide surface and subsequent reaction once bound. It is presumable that complexation of metal ions to amino acid derivatives with large α-substituents will be disfavoured by steric interactions, and evidence for preferential complexation of glycine residues by copper ions has been reported in earlier work.[11] In addition, the relative ease of formation of α-centred glycine radicals *via* hydrogen atom abstraction[8] presumably contributes to the selectivity for reaction of glycine residues in the dipeptides **3a – c** and **5a,b**.

Nickel peroxide provides methodology for selective oxidative cleavage of glycine residues in dipeptides, which is analogous to the process catalysed in biology by peptidylglycine α-amidating monooxygenase (PAM).[12,13] This enzyme complex[13] is responsible for posttranslational activation of many peptide hormones and neuropeptides, through reaction of glycine-extended precursors to give *C*-terminal amides (*Scheme 4*). Both the nickel peroxide reaction and the enzyme catalysed process involve α-hydrogen atom transfer from the reactive centre[14] and proceed *via* formation of an α-hydroxy amino acid intermediate.[15] The glycine selectivity displayed by nickel peroxide mirrors that of PAM[16] and the factors that contribute to this selectivity may similarly contribute to the substrate selectivity displayed by PAM. It is likely that the natural substrates of PAM are synthesised with a *C*-terminal glycine residue because that residue is so easily removed by oxidative cleavage, and this process presumably provides the most efficient route available in biology for the synthesis of



Scheme 4

peptidyl amides. Nickel peroxide serves as a chemical model for PAM, and this model has potential applicability in the development of enzyme inhibitors for the control of metabolic disorders associated with overproduction of peptide hormones.

EXPERIMENTAL

General. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were recorded on a Jasco IRA-1 spectrophotometer as nujol mulls between sodium chloride plates, or as solutions as indicated. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were recorded on either a Bruker ACP 300 or CXP 300 spectrometer as dilute solutions in deuterochloroform, using tetramethylsilane as internal standard. Electron impact mass spectra and high resolution mass spectra were recorded on an AEI MS-3010 spectrometer, using an ionising voltage of 70 eV. Elemental analyses were performed by Canadian Microanalytical Service Ltd., New Westminster, British Columbia, Canada. Preparative thin layer chromatographies were carried out on a Chromatotron 7924T (Harrison Research, Palo Alto / TC Research, Norwich) using Merck silica gel $60_{PF\text{-}254}$ (Art. 7749). All organic extracts were dried over anhydrous magnesium sulphate. Light petroleum refers to the fraction with b.p. 66 – 69 °C.

Nickel peroxide was prepared according to the method of Nakagawa *et al.*,[1] and its available oxygen content was determined as 2.9×10^{-3} mol g^{-1}. α,α-Dideuteroglycine was prepared by treatment of glycine with acetic anhydride / D_2O.[17] The amino acid and dipeptide derivatives **1a**, **3a – c**, **5a – d**, **11** and **12** used in this work were prepared from the corresponding amino acids using standard procedures. Of these compounds **1a**, **3a – c**, **5a,b,d**, **11** and **12** had spectroscopic properties and physical constants in agreement with those previously reported,[8,–10,16,18–22] whereas **5c** was fully characterised, as described below.

General Procedure for Nickel Peroxide Oxidations of Amino Acid and Dipeptide Derivatives. Typically a solution of the amino acid or dipeptide derivative (100 - 200 mg) in benzene (20 ml) was treated with nickel peroxide (2 – 4 mole equiv.) at reflux under nitrogen for 2 – 4 hr. The heterogeneous reaction mixture was filtered on diatomaceous earth whilst hot, to remove nickel salts, and the filtrate was concentrated under reduced pressure. The products of reaction were isolated *via* preparative thin layer chromatography of the residue, eluting with a mixture of light petroleum and ethyl acetate (*Tables 1 and 2*). The products of these reactions, **4a – c**, **8a – c** and **10c,d**, were either fully characterised, as described below, or had spectroscopic properties and physical constants in agreement with those previously reported.[9,23–26]

Table 2. Reaction of *N*-benzoylsarcosine methyl ester (**12**) and *N*-methylbenzamide (**13**) with nickel peroxide.

Substrate	Product	Yield	Corrected Yield†
12	**13**	25%	35%
	2	7%	10%
13	**2**	22%	50%

† based on recovered starting material.

N-*Phthaloyl-(*S*)-phenylalanyl-(*R,S*)-aspartic Acid Dimethyl Ester (**5c**).* *N*-Phthaloyl-(*S*)-phenylalanine,[26] prepared in a melt reaction[27] between phthalic anhydride and (*S*)-phenylalanine, was coupled with (*R,S*)-aspartic acid dimethyl ester hydrochloride *via* the mixed anhydride formed upon treatment with ethyl chloroformate. Chromatography of the crude product gave *N*-phthaloyl-(*S*)-phenylalanyl-(*R,S*)-aspartic acid dimethyl ester (**5c**) as a colourless oil, the diastereomers of which were separated by fractional crystallisation from methanol.

N-Phthaloyl-(*S*)-phenylalanyl-(*R,S*)-aspartic acid dimethyl ester (**5c**), first diastereomer: m.p. 110 – 115 °C; IR (nujol) 3525, 3370, 3028, 2950, 1780, 1710, 1620, 1524, 1443, 1386, 1220, 1100, 1000, 918, 880, 800, 720, 700 cm^{-1}; ^1H NMR δ 2.92 (1H, dd, *J* 17.2, 4.4 Hz), 2.98 (1H, dd, *J* 17.2, 4.4 Hz), 3.58 (2H, m), 3.68 (3H, s), 3.71 (3H, s), 4.85 (1H, dt, *J* 9.2, 4.4 Hz), 5.14 (1H, dd, *J* 9.3, 7.3 Hz), 7.09 (1H, broad d, *J* 9.2 Hz), 7.16 (5H, m), 7.55 (2H, m), 7.80 (2H, m); ^{13}C NMR δ 171.36, 170.79, 168.30, 167.76, 136.59, 134.24, 131.34, 128.89, 126.84, 128.57, 123.45, 55.07, 52.83, 52.04, 48.89, 35.77, 34.58; MS *m/z* (relative intensity) 438 (M^+, 19), 437 (4), 436 (2), 370 (1), 292(5), 291 (7), 278 (8), 277 (11), 251 (31), 250 (100), 249 (57), 233 (12), 232 (76), 160 (40), 132 (15), 131(76), 130(12); HRMS calcd for $C_{23}H_{22}N_2O_7$ *m/z* 438.1427 (M^+), found 438.1441; Anal. Calcd for $C_{23}H_{22}N_2O_7$: C, 63.01; H, 5.06; N, 6.39. Found: C, 63.11; H, 5.08; N, 6.45.

N-Phthaloyl-(*S*)-phenylalanyl-(*R,S*)-aspartic acid dimethyl ester (**5c**), second diastereomer: m.p. 94 – 97 °C; IR (nujol) 3525, 3370, 3028, 2950, 1780, 1710, 1620, 1524, 1443, 1386, 1220, 1100, 1000, 918, 880, 800, 720, 700 cm^{-1}; ^1H NMR δ 2.94 (1H, dd, *J* 17.3, 4.4 Hz), 2.99 (2H, dd, *J* 17.3, 4.4 Hz), 3.49 (2H, m), 3.63 (3H, s), 3.73 (3H, s), 4.88 (1H, dt, *J* 9.2, 4.4 Hz), 5.15 (1H, dd, *J* 10.6, 5.8 Hz), 7.09 (1H, broad d, J 9.2 Hz), 7.13 (5H, m), 7.69 (2H, m), 7.77 (2H, m); ^{13}C NMR δ 171.47, 170.78, 168.07, 167.79, 136.50, 134.27, 131.37, 128.93, 128.59, 126.94, 123.48, 55.21, 52.92, 52.00, 48.84, 35.68, 34.69; MS *m/z* (relative intensity) 438 (M^+, 19), 437 (4), 436 (2), 370 (1), 292 (5), 291 (7), 278 (8), 277 (11), 251 (31), 250 (100), 249 (57), 233 (12), 232 (76), 160 (40), 132 (15), 131(76), 130 (12); HRMS calcd for $C_{23}H_{22}N_2O_7$ *m/z* 438.1427 (M^+), found 438.1441; Anal. Calcd for $C_{23}H_{22}N_2O_7$: C, 63.01; H, 5.06; N, 6.39. Found: C, 62.64; H, 5.14; N, 6.34.

Reaction of N-*Phthaloyl-(*S*)-phenylalanyl-(*R,S*)-aspartic Acid Dimethyl Ester (**5c**) with Nickel Peroxide.* *N*-Phthaloyl-(*S*)-phenylalanyl-(*R,S*)-aspartic acid dimethyl ester (**5c**) (30 mg, 0.07 mmol) in benzene (5 ml) was treated with nickel peroxide (4 mole equiv.) at reflux under nitrogen overnight. Workup and chromatography of the reaction mixture afforded the didehydroaspartate **10c**, the amide **8c** and unreacted starting material **5c** (11 mg, 37%).

N-Phthaloyl-(*S*)-phenylalanyl-α,β-didehydroaspartic acid dimethyl ester (**10c**), as an oil (5 mg, 17%): IR ($CDCl_3$) 3320, 3288, 3028, 2952, 1800, 1740, 1710, 1660, 1500, 1480, 1438, 1400, 1396, 1310, 1240,

1200, 1145, 1115, 1100, 1040, 980, cm^{-1}; ^1H NMR δ 3.61 (2H, m), 3.63 (3H, s), 3.86 (3H, s), 5.26 (1H, t, *J* 8.3 Hz), 5.58 (1H, s), 7.19 (5H, s), 7.70 (2H, m), 7.78 (2H, m), 10.75 (1H, broad s); ^{13}C NMR δ 167.96, 167.40, 166.77, 163.78, 142.84, 136.02, 134.3, 131.32, 128.86, 128.61, 127.03, 123.60, 103.29, 55.00, 53.16, 51.99, 33.96; MS *m/z* (relative intensity) 436 (M^+, 4), 406 (1), 405 (2), 404 (1), 378 (5), 377 (14), 345 (2), 317 (1), 287 (4), 251 (21), 250 (100), 249 (67), 232 (28), 230 (8), 229 (12), 174 (6), 160 (4), 147 (6); HRMS calcd for $C_{23}H_{20}N_2O_7$ *m/z* 436.1270 (M^+), found 436.1276.

N-Phthaloyl-(*S*)-phenylalaninamide (**8c**), recrystallised from ethanol as colourless crystals (4 mg, 21%): m.p. 228 – 230 °C (lit.[26] 229 – 230 °C); ^1H NMR δ 3.56 (2H, m), 5.13 (1H, dd, *J* 9.1, 7.7 Hz), 5.50 (1H, broad s), 6.12 (1H, broad s), 7.19 (5H, m), 7.71 (2H, m), 7.79 (2H, m); MS *m/z* (relative intensity) 294 (M^+, 39), 292 (4), 278 (7), 277 (15), 251 (22), 250 (100), 249 (72), 233 (17), 232 (78), 160 (33), 147 (78); HRMS calcd for $C_{17}H_{14}N_2O_3$ *m/z* 294.1004 (M^+), found 294.1018.

Reaction of N-*Phthaloylglycyl-(*R,S*)-aspartic Acid Dimethyl Ester (**5d**) with Nickel Peroxide.* *N*-Phthaloylglycyl-(*R,S*)-aspartic acid dimethyl ester (**5d**)[9,21] (30 mg, 0.09 mmol) in benzene (5 ml) was treated with nickel peroxide (4 mole equiv.) at reflux under nitrogen overnight. Workup and chromatography of the reaction mixture afforded the didehydroaspartate **10d** and unreacted starting material **5d** (11 mg, 37%).

N-Phthaloylglycyl-α,β-didehydroaspartic acid dimethyl ester (**10d**), recrystallised from ethyl acetate / light petroleum as colourless needles (16 mg, 54%): m.p. 179 – 181 °C (lit.[9] 175 – 176 °C); IR ($CHCl_3$) 3288, 2952, 1788, 1728, 1694, 1640, 1438, 1420, 1396, 1290, cm^{-1}; ^1H NMR δ 3.76 (3H, s), 3.82 (3H, s), 4.53 (2H, s), 5.60 (1H, s), 7.76 (2H, m), 7.90 (2H, m), 10.54 (1H, broad s); ^{13}C NMR δ 168.18, 167.33, 164.40, 163.62, 142.78, 134.35, 131.94, 123.77, 102.97, 53.15, 52.10, 40.71; MS *m/z* (relative intensity) 346 (M^+, 5), 315 (9), 288 (72), 256 (5), 186 (24), 161 (39), 160 (100); HRMS calcd for $C_{16}H_{14}N_2O_7$ *m/z* 346.0801 (M^+), found 346.0791.

Reaction of N-*Benzoyl-α,α-dideuteroglycine Methyl Ester (**11**) with Nickel Peroxide.* *N*-Benzoyl-α,α-dideuteroglycine methyl ester (**11**)[8] (80% $^2H_2$, 18% $^2H_1$ by mass spectrometry, 50 mg, 0.26 mmol) in benzene (10 ml), was treated with nickel peroxide (2.6 mole equiv.) at reflux under nitrogen for 1 hr. Workup and chromatography of the reaction mixture afforded benzamide (**2**) (6 mg, 19%) and unreacted starting material **11** (82% $^2H_2$, 13% $^2H_1$, 35 mg, 70%).

Relative Rate of Reaction of N-*Benzoylglycine Methyl Ester (**1a**) and* N-*Benzoyl-α,α-dideuteroglycine Methyl Ester (**11**), with Nickel Peroxide.* A mixture of **1a**[18] (50 mg, 0.26 mmol) and **11** (50 mg, 0.26 mmol) with *N-tert*-butylbenzamide (25 mg, 0.15 mmol) as internal standard, in benzene (10 ml), was treated with nickel peroxide (2.6 mole equiv.) at reflux, under nitrogen. Aliquots were removed at intervals and analysed by ^1H NMR spectroscopy following filtration and solvent removal. The initial and final relative ratios of the amino acid derivatives **1a** and **11** were determined by peak integration. The relative rate of reaction of **1a** to **11** was calculated using *Equation 1*[8,28] as 2.9 ± 0.5. The error limits quoted represent the sample standard deviation for experiments carried out in triplicate and analyses performed in triplicate.

$$k_X/k_Y = \ln([X]_{t=1}/[X]_{t=0})/\ln([Y]_{t=1}/[Y]_{t=0}) \qquad \textit{Equation 1}$$

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