Synthesis of Structure and Function Diverse α-D-Diazoacetates, α-D-Diazoacetamides, α-D-Diazoketones, and the Antibiotic α-D-Azaserine

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Abstract: Using 0.1 mol% to 1 mol% potassium carbonate in an acetonitrile–deuterium oxide mixture acts as a 'privileged' reaction system, which at ambient temperature affords, via a one-pot–one-cycle procedure, α-D-diazoacetates, α-D-diazoacetamides, or α-D-diazoketones from the corresponding nondeuterated form. The protocol is inexpensive, employs readily available materials, does not require harsh reaction conditions, requires two hours for completion, and affords the desired products in good yields and with excellent levels of deuterium incorporation. Exemplifying our protocol the first isotope labelled synthesis of *N*-Boc-α-D-azaserine with ≥95% D-incorporation is reported.

Key words: isotope, deuterium-labelled compounds, diazoester, azaserine

Deuterium-labelled compounds are very important entities widely employed in analytical, biological, organic, material, medicinal and physical organic chemistry.[1] Exemplifying their utility, they are used for the analysis of agrochemicals and drug metabolites,[2] the development of sensitive mass spectrometry and NMR spectroscopy probes,[3] used in reaction mechanism and kinetic studies, and employed within new innovative materials i.e. optical fibres, non-linear optics and OLEDs.[4] An interesting recent application has focused on generating selectively deuterated (at metabolic 'soft spots') pharmaceuticals and investigating their potential to display enhanced metabolic stability.[5]

Diazocarbonyl species are also important chemical entities with an impressive chemical 'pedigree'.[6] Structure and function diverse isotope labelled i.e. D-, ^{13}C-, ^{14}C-, or ^{15}N-α-diazoesters have found applications in agrochemical, medicinal, and mechanistic synthetic chemistry. Substantiating the general importance of isotope-labelled α-diazoesters (Scheme 1), Unny et al. executed the synthesis of ^{14}C-labelled D-*threo*-chloramphenicol (**1**) using, as the key starting material, ethyl [1-^{14}C]-dichloroacetate (generated by passing Cl_2 through a solution of ethyl [1-^{14}C]-diazoacetate).[7] Johnson required ^{14}C-labelled γ-cyhalothrin (the unlabelled version is an active constituent of the pyrethroid family of insecticides) for metabolism and environmental studies, a key ^{14}C-labelled γ-cyhalothrin intermediate employed 1-(1,1-dichloro-2,2,2-trifluoroethyl)-3-methylbut-2-enyl ^{14}C-diazoester **3** as the starting material.[8] Mezzetti et al. generated optically active N-benzhydryl ^{13}C- and ^{15}N-aziridines by reacting *N*-benzhydryl imines with ^{13}C-**5** or ^{15}N-**6** in the presence of catalytic quantities of RuCl(PNNP)PF_6 [PNNP = (1*R*,2*R*)-*N*,*N*-bis[2-(diphenylphosphino)benzyl]cyclohexane-1,2-diamine)] allowing them to investigate and probe mechanistic aspects surrounding aziridine formation.[9] Baldwin et al. used labelled alkyl α-D-diazoacetates, e.g. **5**, to probe enzyme mechanisms,[10] to investigate thermal stereomutations,[11] and for the asymmetric synthesis of deuterated cyclopropanes using deuterated **2**.[12] The *tert*-butyl ester analogue of **4** was employed by Golding et al. for the synthesis of deuterated cyclopropanes.[13] Shafer et al. used 4-nitrophenyl ^{14}C-diazoacetate for its attachment to chymotrypsin[14] and Galletti et al. synthesised 4-(1-deuteroalkylidine)azetidin-2-one **7** in 51% yield by reacting benzyl ester **9** with 3-acetoxyazetidin-2-one and titanium(IV) chloride.[15] The copper-promoted cycloaddition of acetylides with α-D-diazocarbonyl compounds generating pyrazoles was reported by Ready et al. who reacted **8** with α-deuterated α-diazo benzyl ester (95% D-incorporation) **9** in the presence of a copper(I) cyanide–lithium chloride complex, affording pyrazole **10** with a slightly disappointing 73% D-incorporation.[16] Finally, Nakanishi et al. reported the synthesis and applications of 3-([1-^{14}C]diazoacetoxy)-*trans*-retinal as a photoaffinity label of bacteriorhodopsin.[17]

We required a generic, efficient, straightforward, high yielding synthesis of α-D-diazoesters, α-D-diazoamides, and α-D-diazoketones (Figure 1).[18] Critical for our application these had to be afforded with excellent and reproducible levels of D-incorporation.

H/D Exchange at a carbon atom is probably the most important synthetic route to high value (both chemical and monetary) deuterated compounds. In some instances H/D exchange proceeds without the need for a catalyst. However in many cases non-catalysed H/D-exchange reactions utilise high temperatures e.g. 100–400 °C;[19] sealed and pressurised reactors (using expensive D_2); a stoichiometric quantity of a strongly basic[20] or acidic reagent;[21] or in conjunction with D_2 require expensive platinum or pyrophoric Raney nickel. A consequence of these aggressive reagent/reaction conditions is an imposed chemical limitation on the types of substrates amenable to H/D exchange, a fact that precludes substrates, e.g. α-diazoesters, that are sensitive to high temperatures or strongly basic or acidic reaction conditions.

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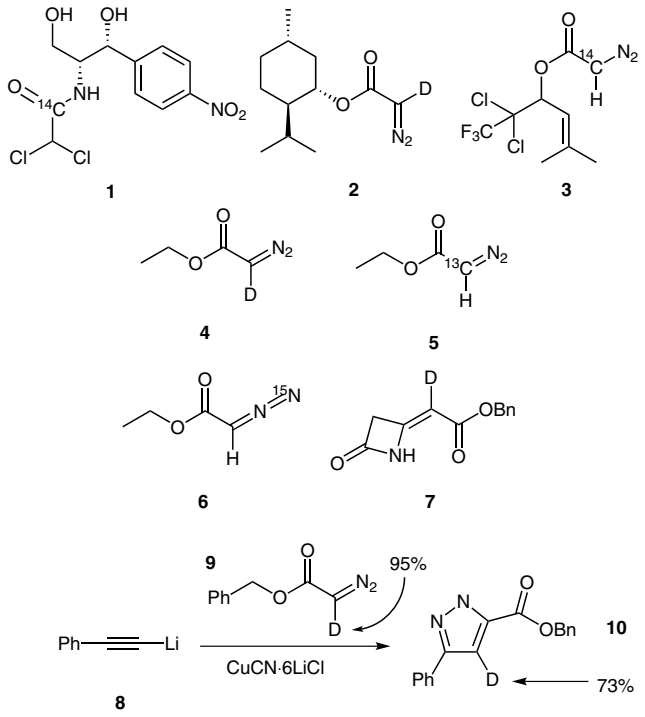


Scheme 1 Reported α-diazoesters and the application of deuterated α-diazoester **9** in the synthesis of **10**

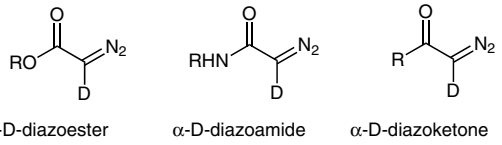


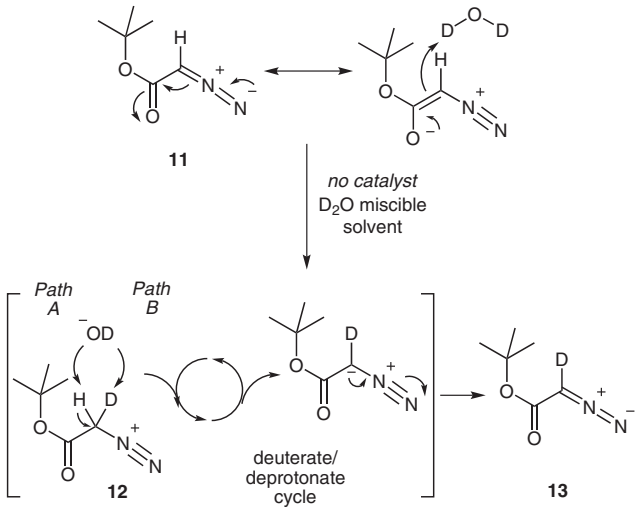
Figure 1 Generic α-D-diazoesters, α-D-amides, and α-D-ketones

Previous syntheses of α-D-diazocarbonyl species have focused on α-D-diazoacetates (no examples of α-D-diazoacetamides are in the literature) via H/D-exchange reactions requiring multiple exposures to excess deuterium oxide (using 99.8% and 100 atom% D_2O) and strong base (i.e., NaOD); a phase-transfer reagent (e.g., CTAB);[22] or biphasic reaction conditions requiring multiple exposures to deuterium oxide.[23] These protocols require from two-to-three cycles of 12–48 hour reaction times, a fact that results in accumulated reaction times of 24–144 hours.

The possibility that stirring **11** in a deuterium oxide miscible solvent with deuterium oxide may afford a convenient route to **13** intrigued us. Thus stirring deuterium oxide with the enolate of **11** generating in situ a deuteroxide anion (Scheme 2) that chemoselectively deprotonates the weaker C–H bond on **12**, i.e. Path A (due to lower zero-point energies C–D bonds are approximately 3 kJ mol^{-1} stronger than C–H bonds[24]), affording, via successive deuterate/deprotonate cycles, increased levels of deuterium in **13**.

A limited number of relatively inexpensive deuterated solvents are available, examples include deuterated chloroform, acetone, methanol, acetonitrile, and dimethyl sulfoxide. Using these deuterated solvents we may be able to readily monitor the exchange reaction of **11** (α-H-diazoester → α-D-diazoester) in 'real time' by ^{1}H NMR spectroscopy as it proceeds to **13**. Employing acetone-d_6 (D_6 99.9%) as an inexpensive, deuterated, nontoxic, deuterium oxide miscible solvent and **11** allowed ^{1}H NMR spectroscopic monitoring of the reaction via loss of the α-diazoacetate singlet at δ = 4.80. The H/D-exchange reaction was effective affording **13** with 80% D-incorporation after only 2.5 hours.

Partially deuterium oxide miscible solvents i.e. *tert*-butyl methyl ether, diethyl ether, ethyl acetate, or methyl ethyl ketone (Table 1) were not particularly effective, affording moderate levels, i.e. 42–55%, of D-incorporation in **13**. Switching to a completely deuterium oxide miscible solvent generally had a positive effect on the H/D exchange, methanol-d_4 (D_4 99.8%) afforded 80% D-incorporation, propan-2-ol however afforded a lower 59% D-incorporation. Cyclic ethers such as dioxane and tetrahydrofuran also afforded lowered levels of D-incorporation i.e. 47% and 46% respectively (values broadly similar to those of partially miscible solvents). Using ^{1}H NMR spectroscopy as our diagnostic tool the application of polar aprotic solvents such as dimethyl sulfoxide-d_6 (D_6 99.8%), *N*,*N*-dimethylformamide, and acetonitrile-d_3 (D_3 99.8%) was investigated, α-D-**13** was afforded with variable levels of D-incorporation i.e. 76%, 79% and 59% respectively. Thus utilising a deuterium oxide miscible solvent conferred significant rate benefits on the H/D-exchange reaction. Albeit the levels of D-incorporation were reasonable for such a simple procedure, they were not exemplary. Investigating higher reaction temperatures (30 °C and 35 °C) on the level of H/D exchange in **11** → **13** found no significant positive or negative effect.



Scheme 2 Uncatalysed H/D-exchange reaction generating **13**

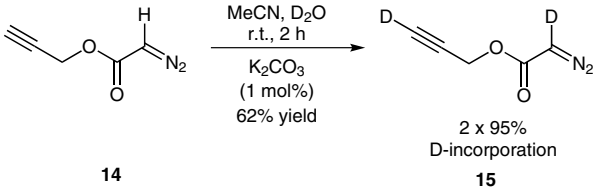
Exploiting the use of acetonitrile-d_3 (allowed 'real time' ^{1}H NMR spectroscopic monitoring of **11** to **13**) as our preferred solvent,[25] we considered dovetailing it with a catalytic amount of weak base dissolved in deuterium oxide (100 equiv). We were delighted that employing **11** and 10 mol% potassium carbonate in a homogenous mixture of deuterium oxide–acetonitrile-d_3 (1:1) gave, after only two hours, **13** in 45% yield, and, most importantly, with ≥95% D-incorporation. Reducing the catalyst to 1 mol% or 0.1 mol% afforded **13** in two and four hours in 72% and 53% yields and with impressive levels of D-incorporation, i.e. ≥95% and 86%, respectively. The lower isolated yield of **13** using 0.1 mol% potassium carbonate for the longer four-hour reaction time was attributed to **12** reacting with the deuterium oxide affording *tert*-butyl glycolate which, being more water soluble, was not extracted into dichloromethane.

Table 1 Solvent Study on H/D Exchange and Synthesis of **13** Using a Mixture of Deuterium Oxide and Solvent[a]

Solvent	D_2O miscibility (%)	D-Incorporation (%)[c]
t-BuOMe	5	42
Et_2O	7	51
EtOAc	9	46
MEK	24	55
acetone-d_6[b]	100	80
CD_3OD[b]	100	80
i-PrOH	100	59
dioxane	100	47
THF	100	46
DMSO-d_6[b]	100	76
DMF	100	79
CD_3CN[b]	100	59

[a] Reaction conditions: **11** (1 mmol), solvent (0.5 mL), D_2O (0.5 mL), 23 °C, 2.5 h.
[b] Deuterated solvents were employed in these examples for direct ^{1}H NMR analysis used to establish the levels of deuterium incorporation.
[c] Levels of deuterium were established by removing the solvent, redissolving in $CDCl_3$ and integrating the singlet at 4.80 ppm.

We investigated the levels of D-incorporation and yield of **13** when reduced amounts of deuterium oxide were employed i.e. 10, 20, and 33 equivalents in the presence of **11**, 1 mol% potassium carbonate, and undeuterated HPLC-grade acetonitrile over a two-hour reaction period. Utilising 10 equivalents of deuterium oxide gave **13** in 63% yield with 79% D-incorporation, increasing to 20 equivalents of deuterium oxide afforded **13** in 59% yield and an increased 90% D-incorporation. When 33 equivalents of deuterium oxide were employed, **13** was obtained in 65% yield with a further increased D-incorporation of 95%. With the solvent study complete and confident that the H/D-exchange reactions would proceed without the need for deuterated acetonitrile, we wanted to exploit and exemplify our results using 1 mol% potassium carbonate as an extremely inexpensive 'off-the-shelf' catalyst. Dissolving it in deuterium oxide (99.9% D_2O, 100 equiv), this solution was added to **14** dissolved in acetonitrile.[26] After two hours at ambient temperature and only one cycle of deuterium oxide, **14** was transformed into **15** with ≥95% D-incorporation (Scheme 3). As previously reported, we observed the α-diazoester H/D exchange of the terminal proton on the alkyne of **14** underwent H/D exchange with ≥95% D-incorporation.[27]



14 **15**

Scheme 3 Synthesis of double isotope labelled **15**

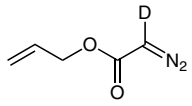
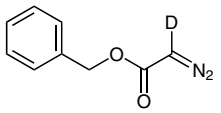
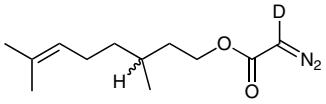
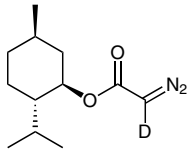
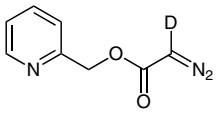
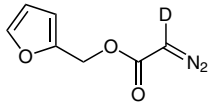
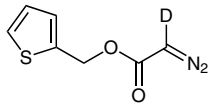
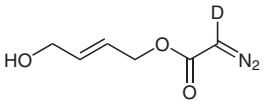
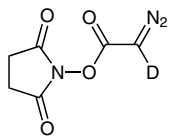
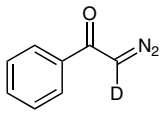
Employing deuterium oxide (99.9 atom% D, 100 equivalents) and 1 mol% potassium carbonate a variety of structurally diverse α-diazoacetates[28] were screened for their transformation into the corresponding deuterated forms. The results from this study are outlined in Table 2.

Badet et al. reported the synthesis of undeuterated 2,5-dioxopyrrolidin-1-yl diazoacetate and its ability to diazoacetylate efficiently amines, phenols, thiophenols, or peptides.[29] Reacting base-sensitive undeuterated 2,5-dioxopyrrolidin-1-yl diazoacetate under our deuteration conditions generated α-D-**24** in 72% yield and ≥95% D-incorporation. Although the isolated yields were on the whole acceptable, i.e. **19** (89%) and **20** (90%), the isolated yields of, for example **21** (67%) and **24** (72%) were slightly lower, this was attributed to the formation of the corresponding glycolate or hydrolysis of the sensitive ester group in **24**. The synthesis of cholesteryl α-D-diazoacetate (not shown) was complicated and problematic. Its lipophilic nature made its dissolution in the deuterium oxide–acetonitrile mixture incomplete, a waxy solid formed that was recalcitrant to H/D exchange. Warming to 35 °C negated the solubility issue, however for reasons that are not yet clear the level of D-incorporation remained low at 49%.

Finally testing an α-D-diazoketone was considered important. Diazoacetophenone was transformed into α-D-**25** with ≥95% D-incorporation and in 85% yield (Table 2). Expanding the substrate scope more complex functional groups were considered important. Reacting succinimidyl diazoacetate with *rac*-phenylalanine methyl ester, (*S*)-leucine methyl ester, (*S*)-proline methyl ester, and *tert*-butyl (*S*)-3-amino-3-phenylpropanoate afforded the corresponding *N*-diazoacetamides in good yields (58–65%).

When subjected to our standard deuteration conditions the desired, previously unreported, α-D-diazoacetamide de-

Table 2 H/D Exchange and D–Incorporation To Give **16**–**25**[a,b]

Product	Yield (%)	D-Incorporation (%)
 **16**	–[c]	≥95
 **17**	74	≥95
 **18**	74	≥95
 **19**	89	≥95
 **20**	82	≥95
 **21**	67	≥95
 **22**	90	≥95
 **23**	76	≥95
 **24**	72	≥95
 **25**	85	≥95

[a] One Cycle of D_2O was used throughout.
[b] Reaction conditions: K_2CO_3 (1 mol%) D_2O–MeCN (1.17:1), r.t., 2 h.
[c] Not determined.

rived amino acids **26**–**29** (Figure 2) were afforded in 71–84% yields and superb levels of D-incorporation, i.e. all ≥95%. Furthermore comparing the optical rotation ($[\alpha]_D$) values of the undeuterated precursors methyl *N*-diazoacetyl-L-leucinate (–6.3) and methyl *N*-diazoacetyl-L-prolinate (–114.9) with the corresponding deuterated forms, i.e. (*S*)-**27** (–6.0) and (*S*)-**28** (–124.7) indicated that during the H → D exchange process no racemisation of the stereogenic centre within the α-amino acids had taken place.

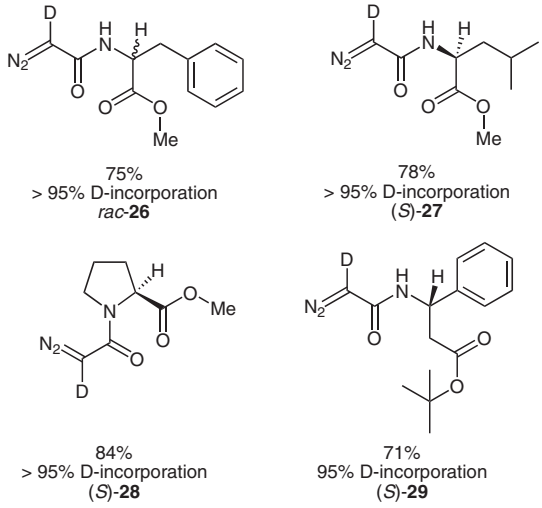
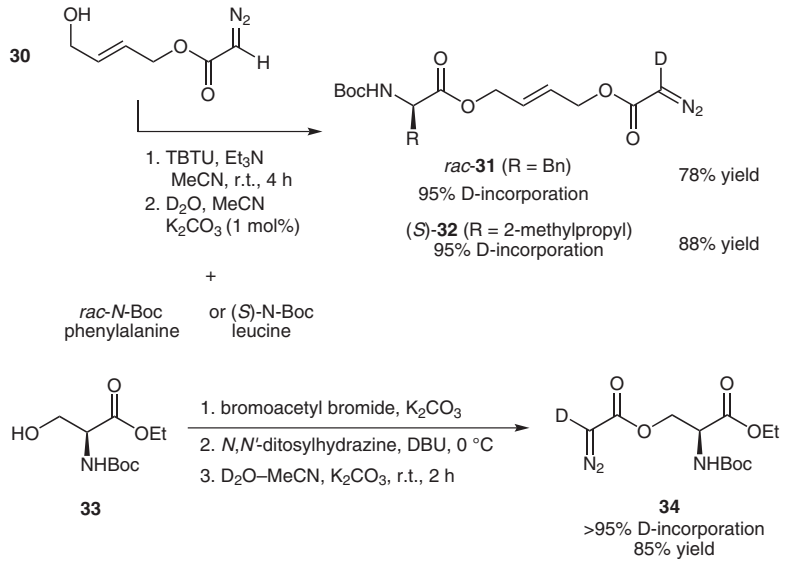


Figure 2 α-D-Diazoacetamide-derived α- and β-amino acids **26**–**29**

Employing *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), **30**, and *rac*-*N*-Boc-phenylalanine or (*S*)-*N*-Boc-leucine afforded *rac*-(*E*)-4-(diazoacetoxy)but-2-enyl 2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoate or (*S*)-(*E*)-4-(diazoacetoxy)but-2-enyl 2-[(*tert*-butoxycarbonyl)amino]-4-methylpentanoate, respectively, which when subjected to our standard deuteration conditions afforded *rac*-**31** and (*S*)-**32** (Scheme 4) with superb levels of deuterium incorporation (both ≥95%) and in excellent yields (78% and 88% yields, respectively).

Azaserine is the most widely studied naturally occurring diazo compound with over 1100 publications.[30] Isolated (*Streptomyces fragilis*) as a pale yellow-green crystalline solid in 1954[31] it has antitumour as well as antibiotic properties and, as an analogue of (*S*)-glutamine, it functions as a competitive inhibitor of enzymes that use (*S*)-glutamine. The cytotoxic effects of azaserine have been attributed to inhibition of purine synthesis, primarily via inhibiting conversion of formylglycinamide ribotide into formylglycinamidine ribotide through alkylation of a cysteine residue in the active site of phosphoribosyl formylglycinamidine synthetase.[32]

However recent structural studies with the δ-glutamyltranspeptidase azaserine complex have indicated that glutamine antagonists may act via an alternative mechanism.[33] A large number of studies on the antitumour properties of azaserine have been conducted, these have been reviewed.[34] Although a number of clinical trials



Scheme 4 Synthesis of *rac*-**31**, (*S*)-**32**, and (*S*)-*N*-Boc-α-D-azaserine ethyl ester **34**

have been performed using azaserine with some showing useful levels of activity in leukaemia, azaserine was less effective than other agents, and as a result, clinical studies on the compound have been abandoned.

The widespread use and application of isotope-labelled pharmaceuticals and NCEs make the synthesis of an isotope-labelled azaserine analogue highly desirable. Reacting (*S*)-**33** using Fukuyama reaction conditions allowed the subsequent α-diazodeuteration to be investigated. Employing our standard isotope incorporating conditions the synthesis of ethyl (*S*)-*N*-(Boc)-3-(α-D-diazoacetoxy)propanoate **34** was achieved with ≥95% D-incorporation in only two hours (Scheme 4). This represents the first synthesis of a deuterium-labelled analogue of the important antibiotic azaserine.

A straightforward, durable, efficient deuteration protocol amenable to the production of structurally diverse α-D-diazoacetates, α-D-diazoacetamides, and α-D-diazoketones has been developed. Our method is quick, uses an off-the-shelf catalyst; it requires neither specialist equipment nor rigorously dried solvent; is conducted at ambient temperature and proceeds via a one-cycle–one-pot reaction using inexpensive, readily available deuterium oxide (≥95% isotope inclusion). Generating the isotope-labelled compounds via a single cycle rather than the conventional 'multiple work up, analysis, resubmit regimes' affords significant cost and time savings.

We have exemplified the broad utility of this procedure and its applicability to functionalised systems by synthesising deuterated *N*-diazoacetamide-derived racemic and optically active α- and β-amino acids. Furthermore the utility of the protocol within isotope derived natural product synthesis has been demonstrated via the efficient formation of a *N*-Boc-protected analogue of biologically active (*S*)-α-D-azaserine. The ease of conducting these reactions, their efficiency and the excellent levels of deuterium incorporation into structurally diverse substrates will allow this protocol to have widespread application in synthetic, biological, agrochemical, medicinal, and pharmaceutical chemistry laboratories alike.

MeCN UpS ultra gradient and D_2O (99.9 atom% D) were purchased from ROMIL (Cambridge, UK) and Sigma-Aldrich, respectively, and used as supplied. NMR solvents were purchased from Apollo Scientific Limited and used as supplied. $CDCl_3$ was filtered through basic alumina into a bottle wrapped in Al foil and stored in a dry box. All products were characterised by ^1H and ^{13}C NMR spectroscopy, FT-IR and HRMS. ^1H and ^{13}C NMR data were recorded on 300 MHz and 400 MHz spectrometers and unless otherwise stated with CD_3CN or $CDCl_3$ as solvent referenced to the residual solvent signal [CD_3CN: δ = 1.94 (^1H), δ = 1.39 and 118.69 (^{13}C); $CDCl_3$: δ = 7.26 (^1H), δ = 77.16 (^{13}C)]. Melting points were recorded using open capillary tubes on melting point apparatus and are uncorrected.

The following compounds were prepared by published procedures and in each case the spectral data matched those previously reported: benzyl diazoacetate (0.75 g, 4.26 mmol, 46%);[28] prop-2-ynyl diazoacetate (0.55 g, 4.43 mmol, 25%);[28] (1*R*,2*S*,5*R*)-(–)-menthyl diazoacetate (0.72 g, 3.21 mmol, 50%);[34] diazoacetophenone (1.04 g, 7.12 mmol, 44%);[34] allyl diazoacetate (1.04 g, 8.25 mmol, 48%);[35] cholesteryl diazoacetate (0.3 g, 0.66 mmol, 25%).[36]

rac-3,7-Dimethyloct-6-enyl Diazoacetate[28]
Yellow liquid; yield: 0.45 g (2.01 mmol, 32%).

IR (ATR): 3190, 2914, 2106, 1690, 1455, 1358, 1237, 1180, 1054, 740 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.01 (t, *J* = 9 Hz, 1 H), 4.65 (s, 1 H), 4.18–4.06 (m, 2 H), 1.98–1.83 (m, 2 H), 1.69–1.03 (m, 11 H), 0.84 (d, *J* = 9.0 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 131.6, 124.7, 63.6, 46.3, 37.1, 35.8, 29.6, 25.9, 25.6, 19.6, 17.8.

MS (MALDI-TOF): *m*/*z* = 242.2 [M + NH_4].

HRMS (HCIP): *m*/*z* [M + NH_4] calcd for $C_{12}H_{24}N_3O_2$: 242.1863; found: 242.1862.

Furan-2-ylmethyl Diazoacetate[28]
Yellow liquid; yield: 0.86 g (5.18 mmol, 51%).

IR (ATR): 3118, 2106, 1681, 1379, 1344, 1231, 1150, 999, 736 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.39 (dd, *J* = 2, 0.8 Hz, 1 H), 6.39 (d, *J* = 3.2 Hz, 1 H), 6.33 (dd, *J* = 3.2, 1.6 Hz, 1 H), 5.11 (s, 2 H), 4.75 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 149.6, 143.5, 111.0, 110.8, 58.4, 46.6.

MS (MALDI-TOF): *m*/*z* = 166.9 [M].

HRMS (HEIP): *m*/*z* [M] calcd for $C_7H_6N_2O_3$: 166.0373; found: 166.0372.

Pyridin-2-ylmethyl Diazoacetate[28]
Yellow liquid; yield: 0.61 g (3.44 mmol, 38%).

IR (ATR): 3096, 2106, 1680, 1592, 1385, 1348, 1230, 1170, 995, 737 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 8.53 (d, *J* = 4.1 Hz, 1 H), 7.64 (td, *J* = 7.7, 1.8 Hz, 1 H), 7.28 (d, *J* = 7.8 Hz, 1 H), 7.20–7.12 (m, 1 H), 5.25 (s, 2 H), 4.81 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 155.9, 149.7, 137.0, 123.1, 121.9, 67.2, 46.7.

MS (MALDI-TOF): *m*/*z* = 200.3 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_8H_7N_3NaO_2$: 200.0430; found: 200.0425.

Thiophen-2-ylmethyl Diazoacetate[28]
Yellow liquid; yield: 0.71 g (3.90 mmol, 45%).

IR (ATR): 3108, 2106, 1681, 1385, 1339, 1229, 1155, 1001, 834 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.30 (dd, *J* = 5.1, 1.2 Hz, 1 H), 7.10–7.05 (m, 1 H), 6.96 (dd, *J* = 5.0, 3.5 Hz, 1 H), 5.31 (s, 2 H), 4.75 (s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.7, 137.9, 128.6, 127.1, 126.7, 60.6, 46.6.

MS (MALDI-TOF): *m*/*z* = 204.9 [M + Na].

HRMS (HEIP): *m*/*z* [M] calcd for $C_7H_6N_2O_2S$: 182.0144; found: 182.0142.

Synthesis of (*E*)-4-Hydroxybut-2-enyl Diazoacetate (30)

(*E*)-4-Hydroxybut-2-enyl Bromoacetate[37]
From but-2-ene-1,4-diol as a pale yellow liquid; yield: 1.08 g (5.17 mmol, 65%).

IR (ATR): 3370, 2968, 1730, 1407, 1278, 1160, 1109, 960 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 5.97–5.77 (m, 1 H), 5.62 (m, 1 H), 4.76 (d, *J* = 7.0 Hz, 2 H), 4.26 (d, *J* = 6.5 Hz, 2 H), 3.83 (s, 2 H), 2.13 (s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.4, 134.4, 124.5, 61.7, 58.4, 25.6.

MS (MALDI-TOF): *m*/*z* = 232.8 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_6H_9BrNaO_3$: 232.9607; found: 232.9607.

(*E*)-4-Hydroxybut-2-enyl Diazoacetate (30)[28]
From (*E*)-4-hydroxybut-2-enyl bromoacetate as a yellow liquid; yield: 0.08 g (0.10 mmol, 56%).

IR (ATR): 3374, 2921, 2107, 1671, 1377, 1236, 1176, 1016, 739 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.75 (dtt, *J* = 11.2, 6.2, 1.4 Hz, 1 H), 5.62–5.51 (m, 1 H), 4.98 (s, 1 H), 4.70 (ddt, *J* = 6.7, 1.4, 0.7 Hz, 2 H), 4.16–4.08 (m, 2 H), 2.86 (t, *J* = 5.6 Hz, 1 H, OH).

^{13}C NMR (100 MHz, CD_3CN): δ = 134.4, 124.8, 60.5, 57.8, 46.3.

MS (MALDI-TOF): *m*/*z* = 156.1 [M].

HRMS (HCIP): *m*/*z* [M + H] calcd for $C_6H_9N_2O_3$: 157.0608; found: 157.0609.

(*E*)-4-(Diazoacetoxy)but-2-enyl 2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoate
Synthesised according to published procedures.[38] Purification of the product by chromatography (silica gel, EtOAc–petroleum ether, 3:7) afforded a yellow oil; yield: 0.04 g (0.10 mmol, 73%).

IR (ATR): 2976, 2108, 1687, 1364, 1157, 1020 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.36–7.15 (m, 5 H), 5.86–5.61 (m, 2 H), 5.55 (s, 1 H, NH), 5.00 (s, 1 H), 4.75–4.65 (m, 4 H), 4.39–4.32 (m, 1 H), 3.10 (dd, *J* = 14.0, 5.5 Hz, 1 H), 2.91 (dd, *J* = 13.7, 8.7 Hz, 1 H), 1.35 (s, 9 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 172.1, 161.9, 155.7, 137.4, 129.6, 128.8, 128.7, 127.9, 127.1, 79.3, 60.6, 60.2, 55.3, 46.1, 37.3, 27.6.

MS (MALDI-TOF): *m*/*z* = 442.1 [M + K].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{20}H_{25}N_3NaO_6$: 426.1636; found: 426.1639.

(*S*,*E*)-4-(Diazoacetoxy)but-2-enyl 2-[(*tert*-Butoxycarbonyl)amino]-4-methylpentanoate
Synthesised according to a published procedure.[38] Purification of the product by chromatography (silica gel, EtOAc–petroleum ether, 3:7) afforded a yellow oil; yield: 0.03 g (0.08 mmol, 47%).

$[\alpha]_D^{21}$ –11.9 (*c* 1.0, $CHCl_3$).

IR (ATR): 2958, 2111, 1694, 1366, 1160 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.81–5.66 (m, 2 H), 5.56 (s, 1 H, NH), 4.99 (s, 1 H), 4.76–4.66 (m, 4 H), 4.12 (dd, *J* = 15.5, 7.9 Hz, 1 H), 1.73–1.59 (m, 1 H), 1.51 (t, *J* = 7.2 Hz, 2 H), 1.40 (s, 9 H), 0.91 (t, *J* = 6.6 Hz, 6 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 173.3, 162.0, 155.9, 128.6, 128.0, 79.1, 60.6, 60.3, 52.5, 46.3, 40.5, 27.8, 24.8, 22.4, 21.0.

MS (MALDI-TOF): *m*/*z* = 409.2 [M + K].

HRMS (NSI): *m*/*z* [M + NH_4] calcd for $C_{17}H_{31}N_4O_6$: 387.2238; found: 387.2241.

Succinimidyl Diazoacetate
Synthesised according to a published procedure; yield: 0.416 g (2.27 mmol, 46%); spectral data match those previously reported for this compound.[39]

Methyl *N*-Diazoacetyl-D,L-phenylalaninate; Typical Procedure
To a soln of the amine (117 mg, 0.66 mmol) dissolved in dry CH_2Cl_2 (1.5 mL) in a flame-dried round bottom flask was added anhyd Et_3N (115 μL, 0.82 mmol) under an atmosphere of N_2. The soln was cooled to 0 °C. After stirring for 10 min, a soln of succinimidyl diazoacetate (100 mg, 0.55 mmol) in CH_2Cl_2 (1 mL) was added dropwise via syringe. The yellow soln was allowed to warm to r.t. and stirred for 72 h. The solvent was evaporated in vacuo and purification of the product by chromatography (silica gel, Et_2O–petroleum ether, 3:1) afforded a yellow solid; yield: 83 mg (0.34 mmol, 61%); mp 126–128 °C.

IR (ATR): 3101, 3063, 3028, 2945, 2107, 1740, 1603, 1509, 1443, 1382, 1200, 998 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.37–7.19 (m, 5 H), 6.34 (s, 1 H), 5.05 (s, 1 H), 4.71 (m, 1 H), 3.66 (s, 3 H), 3.13 (dd, *J* = 13.8, 5.7 Hz, 1 H), 2.96 (dd, *J* = 13.9, 8.1 Hz, 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 172.4, 165.6, 137.1, 129.5, 128.7, 127.1, 54.3, 52.0, 46.8, 37.7.

LRMS (ES): 270.0 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{12}H_{13}N_3NaO_3$: 270.0849; found: 270.0850.

***tert*-Butyl (*S*)-3-(Diazoacetamido)-3-phenylpropanoate**
Yellow oil; yield: 33 mg (0.11 mmol, 61%).

$[\alpha]_D^{23}$ –54.7 (*c* 1.5, $CHCl_3$).

IR (ATR): 3277, 3095, 2983, 2938, 2099, 1727, 1619, 1561, 1375, 1251, 1146, 701 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.35–7.16 (m, 5 H), 6.59 (s, 1 H), 5.19 (m, 1 H), 4.96 (s, 1 H), 2.61 (m, 2 H), 1.32 (s, 9 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 169.9, 165.0, 142.2, 128.7, 127.6, 126.8, 80.7, 50.9, 46.8, 42.2, 27.4.

MS (MALDI-TOF): *m*/*z* = 327.9 [M + K].

HRMS (NSI): *m*/*z* [M + H] calcd for $C_{15}H_{20}N_3O_3$: 290.1499; found: 290.1503.

Methyl *N*-Diazoacetyl-L-leucinate
Yellow oil; yield: 101 mg (0.48 mmol, 58%).

$[\alpha]_D^{19}$ –6.3 (*c* 1.0, $CHCl_3$).

IR (ATR): 3105, 2959, 2877, 2100, 1741, 1616, 1536, 1380, 1204, 1148 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 6.40 (s, 1 H), 5.06 (s, 1 H), 4.42 (dd, *J* = 14.8, 7.9 Hz, 1 H), 3.65 (s, 3 H), 1.68–1.48 (m, 3 H), 0.90 (m, 6 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 173.6, 165.8, 52.0, 51.3, 46.8, 40.7, 24.8, 22.4, 21.1.

MS (MALDI-TOF): *m*/*z* = 252.0 [M + K].

HRMS (NSI): *m*/*z* [M + H] calcd for $C_9H_{16}N_3O_3$: 214.1186; found: 214.1187.

Methyl *N*-Diazoacetyl-L-prolinate
Yellow oil; yield: 70 mg (0.35 mmol, 65%).

$[\alpha]_D^{22}$ –114.9 (*c* 0.8, $CHCl_3$).

IR (ATR): 3075, 2961, 2927, 2855, 2107, 1736, 1607, 1413, 1163, 730 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 4.88 (s, 1 H), 4.53 (br s, 1 H), 3.72 (s, 3 H), 3.43 (br s, 1 H), 3.28 (br s, 1 H), 2.25–1.90 (m, 4 H).

^1H NMR (300 MHz, CD_3CN): δ = 5.20 (s, 1 H), 4.39 (br s, 1 H), 3.66 (s, 3 H), 3.44–3.24 (m, 2 H), 2.20 (br s, 1 H), 1.93 (m, 3 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 173.3, 164.4, 59.1, 51.9, 46.5, 29.3, 24.5.

MS (MALDI-TOF): *m*/*z* = 220.9 [M + Na].

HRMS (NSI): *m*/*z* [M + H] calcd for $C_8H_{12}N_3O_3$: 198.0873; found: 198.0873.

***tert*-Butyl α-D-Diazoacetate (13); Typical Procedure for α-D-Diazoacetates, α-D-Diazoacetamides and α-D-Diazoketones**
To a soln of *tert*-butyl α-diazoacetate (50 mg, 0.35 mmol) and K_2CO_3 (0.49 mg, 0.0035 mmol) in MeCN (0.75 mL) was added D_2O (0.64 mL). The resulting mixture was stirred vigorously under N_2 for 2 h at r.t. The soln was then extracted with CH_2Cl_2 (3 × 3 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to afford a yellow liquid; yield: 36 mg (0.25 mmol, 72%); ≥95% D-incorporation.

IR (ATR): 2979, 2104, 1684, 1316, 1148 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 4.80 (residual 1 H), 1.45 (s, 9 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 163.1, 82.2, 28.8.

MS (MALDI-TOF): *m*/*z* = 167.0 [M + Na].

3-D-Prop-2-ynyl α-D-Diazoacetate (15)
Yellow liquid; yield: 31.1 mg (0.25 mmol, 62%); ≥95% D-incorporation (CDN_2, C≡CD).

IR (ATR): 2107, 1680, 1300, 1161, 1026 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.04 (residual 1 H), 4.73 (s, 2 H), 2.78 (residual 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 162.0, 77.9, 77.8, 77.7, 75.7, 75.3, 74.9, 52.0.

MS (MALDI-TOF): *m*/*z* = 151.0 [M + Na].

HRMS (HCIP): *m*/*z* [M + NH_4] calcd for $C_5H_6D_2N_4O_2$: 144.0737; found: 144.0737.

Benzyl α-D-Diazoacetate (17)
Yellow liquid; yield: 37.3 mg (0.21 mmol, 74%); ≥95% D-incorporation.

IR (ATR): 2104, 1679, 1299, 1166, 1003 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.40–7.33 (m, 5 H), 5.17 (s, 2 H), 5.03 (residual 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 162.0, 136.8, 128.8, 128.4, 128.3, 66.3.

MS (MALDI-TOF): *m*/*z* = 197.0 [M + NH_4].

(±)-3,7-Dimethyloct-6-enyl α-D-Diazoacetate (18)
Yellow liquid; yield: 22.2 mg (0.10 mmol, 74%); ≥95% D-incorporation.

IR (ATR): 2912, 2105, 1686, 1307, 1176 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.15–5.07 (m, 1 H), 4.93 (residual 1 H), 4.22–4.09 (m, 2 H), 2.06–1.89 (m, 2 H), 1.67 (s, 3 H), 1.60 (s, 3 H), 1.54–1.10 (m, 5 H), 0.90 (d, *J* = 6.6 Hz, 3 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 163.1, 132.4, 125.9, 64.2, 38.0, 36.7, 30.5, 26.4, 26.2, 20.1, 18.1.

MS (MALDI-TOF): *m*/*z* = 242.3 [M + NH_4].

(1*R*,2*S*,5*R*)-(–)-Menthyl α-D-Diazoacetate (19)
Yellow solid; yield: 44.6 mg (0.20 mmol, 89%); ≥95% D-incorporation; mp 36–38 °C.

$[\alpha]_D^{21}$ –85.0 (*c* 1.0, $CHCl_3$).

IR (ATR): 2954, 2103, 1682, 1302, 1179 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 4.92 (residual 1 H), 4.72 (td, *J* = 10.9, 4.4 Hz, 1 H), 1.90–1.78 (m, 1 H), 1.71–1.63 (m, 2 H), 1.57–1.32 (m, 2 H), 1.17–0.81 (m, 10 H), 0.77 (d, *J* = 7.0 Hz, 3 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 162.0, 74.5, 47.4, 41.3, 34.2, 31.5, 26.5, 23.6, 21.6, 20.2, 16.1.

MS (MALDI-TOF): *m*/*z* = 242.3 [M + NH_4].

Pyridin-2-ylmethyl α-D-Diazoacetate (20)
Yellow liquid; yield: 41.1 mg (0.23 mmol, 82%); ≥95% D-incorporation.

IR (ATR): 2105, 1679, 1302, 1167 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 8.55 (d, *J* = 4.7 Hz, 1 H), 7.77 (td, *J* = 7.7, 1.8 Hz, 1 H), 7.37 (d, *J* = 7.8 Hz, 1 H), 7.32–7.24 (m, 1 H), 5.23 (s, 2 H), 5.09 (residual 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 156.4, 149.6, 137.1, 123.2, 121.7, 66.8.

MS (MALDI-TOF): *m*/*z* = 196.9 [M + NH_4].

HRMS (NSI): *m*/*z* [M + H] calcd for $C_8H_7DN_3O_2$: 179.0674; found: 179.0672.

Furan-2-ylmethyl α-D-Diazoacetate (21)
Yellow liquid; yield: 43.9 mg (0.26 mmol, 67%); ≥95% D-incorporation.

IR (ATR): 2106, 1681, 1301, 1161 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.52–7.49 (m, 1 H), 6.48–6.45 (m, 1 H), 6.43–6.40 (m, 1 H), 5.12 (s, 2 H), 4.99 (residual 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 163.0, 151.1, 144.9, 112.0, 59.1.

MS (MALDI-TOF): *m*/*z* = 186.2 [M + NH_4].

HRMS (HCIP): *m*/*z* [M + NH_4] calcd for $C_7H_9DN_3O_3$: 185.0779; found: 185.0780.

Thiophen-2-ylmethyl α-D-Diazoacetate (22)
Yellow liquid; yield: 45.3 mg (0.25 mmol, 90%); ≥95% D-incorporation.

IR (ATR): 2108, 1679, 1304, 1157 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.43–7.41 (m, 1 H), 7.15–7.13 (m, 1 H), 7.02–7.00 (m, 1 H), 5.32 (s, 2 H), 5.00 (residual 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 138.7, 128.7, 127.5, 127.2, 60.6.

MS (MALDI-TOF): *m*/*z* = 206.1 [M + Na].

(*E*)-4-Hydroxybut-2-enyl α-D-Diazoacetate (23)
Yellow liquid; yield: 38.1 mg (0.24 mmol, 76%); ≥95% D-incorporation.

IR (ATR): 3379, 2105, 1670, 1299, 1172, 1015 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.80–5.71 (m, 1 H), 5.61–5.51 (m, 1 H), 4.98 (residual 1 H), 4.72–4.69 (m, 2 H), 4.15–4.11 (m, 2 H), 2.87 (t, *J* = 5.6 Hz, 1 H, OH).

^{13}C NMR (100 MHz, CD_3CN): δ = 162.0, 134.4, 124.8, 60.5, 57.8.

MS (MALDI-TOF): *m*/*z* = 196.6 [M + K].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_6H_7DN_2NaO_3$: 180.0490; found: 180.0485.

2,5-Dioxopyrrolidin-1-yl α-D-Diazoacetate (24)
Pale yellow solid; yield: 28.8 mg (0.16 mmol, 72%); ≥95% D-incorporation; mp 92–94 °C.

IR (ATR): 2121, 1719, 1303, 1198, 1097 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.45 (residual 1 H), 2.77 (s, 4 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 170.7, 162.0, 25.6.

α-D-Diazoacetophenone (25)
Yellow solid; yield: 43 mg (0.29 mmol, 85%); ≥95% D-incorporation; mp 38–40 °C.

IR (ATR): 2108, 1599, 1571, 1323 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.82–7.77 (m, 2 H), 7.63–7.57 (m, 1 H), 7.52–7.46 (m, 2 H), 6.25 (residual 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 187.4, 137.9, 134.0, 130.0, 127.9.

MS (MALDI-TOF): *m*/*z* = 186.2 [M + K].

HRMS (HCIP): *m*/*z* [M + H] calcd for $C_8H_6DN_2O$: 148.0616; found: 148.0614.

Methyl 2-(α-D-Diazoacetamido)-3-phenylpropanoate (26)
Yellow solid; yield: 27.1 mg (0.11 mmol, 75%); ≥95% D-incorporation; mp 80–82 °C.

IR (ATR): 2099, 1735, 1609, 1529, 1432, 1331, 1206 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.36–7.15 (m, 5 H), 6.34 (s, 1 H, NH), 5.03 (residual 1 H), 4.73–4.66 (m, 1 H), 3.66 (s, 3 H), 3.11 (dd, *J* = 13.9, 5.6 Hz, 1 H), 2.94 (dd, *J* = 13.9, 8.1 Hz, 1 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 172.4, 165.6, 137.1, 129.5, 128.7, 127.1, 54.3, 52.0, 37.7.

MS (MALDI-TOF): *m*/*z* = 271.1 [M + Na].

Methyl (*S*)-2-(α-D-Diazoacetamido)-4-methylpentanoate (27)
Yellow oil; yield: 31.2 mg (0.15 mmol, 78%); ≥95% D-incorporation.

$[\alpha]_D^{21}$ –6.0 (*c* 1.0, $CHCl_3$).

IR (ATR): 2956, 2099, 1739, 1612, 1535, 1337, 1202 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 6.34 (s, 1 H, NH), 5.05 (residual 1 H), 4.48–4.36 (m, 1 H), 3.66 (s, 3 H), 1.73–1.49 (m, 3 H), 0.91 (dd, *J* = 7.6, 6.4 Hz, 6 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 173.6, 165.8, 52.0, 51.3, 40.7, 24.8, 22.4, 21.1.

MS (MALDI-TOF): *m*/*z* = 253.1 [M + K].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_9H_{14}DN_3NaO_3$: 237.1068; found: 237.1068.

Methyl (*S*)-1-(α-D-Diazoacetyl)pyrrolidine-2-carboxylate (28)
Yellow oil; yield: 21.9 mg (0.11 mmol, 84%); ≥95% D-incorporation.

$[\alpha]_D^{21}$ –124.7 (*c* 1.0, $CHCl_3$).

IR (ATR): 2106, 1737, 1699, 1601, 1405, 1174 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.20 (residual 1 H), 4.38 (d, *J* = 5.0 Hz, 1 H), 3.66 (s, 3 H), 3.40–3.25 (m, 2 H), 2.19 (br s, 1 H), 2.00–1.88 (m, 3 H).

13C NMR (100 MHz, CD_3CN): δ = 173.3, 159.3, 59.1, 51.9, 46.5, 29.3, 24.5.

MS (MALDI-TOF): *m*/*z* = 221.1 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_8H_{10}DN_3NaO_3$: 221.0755; found: 221.0753.

***tert*-Butyl (*S*)-3-(α-D-Diazoacetamido)-3-phenylpropanoate (29)**
Yellow oil; yield: 23.4 mg (0.081 mmol, 71%); ≥95% D-incorporation.

$[\alpha]_D^{21}$ –35.4 (*c* 1.0, $CHCl_3$).

IR (ATR): 2978, 2106, 1691, 1612, 1540, 1314, 1149 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.39–7.23 (m, 5 H), 6.61 (s, 1 H, NH), 5.32–5.24 (m, 1 H), 5.01 (residual 1 H), 2.76–2.59 (m, 2 H), 1.34 (s, 9 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 169.9, 162.0, 142.2, 128.7, 127.6, 126.8, 80.7, 50.9, 42.2, 27.4.

MS (MALDI-TOF): *m*/*z* = 313.1 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{15}H_{18}DN_3NaO_3$: 313.1381; found: 313.1381.

(*E*)-4-(α-D-Diazoacetoxy)but-2-enyl 2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoate (31)
Yellow oil; yield: 18 mg (0.045 mmol, 78%); ≥95% D-incorporation.

IR (ATR): 2976, 2109, 1691, 1364, 1306, 1165, 1023 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 7.34–7.20 (m, 5 H), 5.84–5.63 (m, 2 H), 5.55 (s, 1 H, NH), 5.00 (residual 1 H), 4.73–4.68 (m, 4 H), 4.39–4.31 (m, 1 H), 3.09 (dd, *J* = 14.0, 5.6 Hz, 1 H), 2.91 (dd, *J* = 13.8, 8.9 Hz, 1 H), 1.35 (s, 9 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 172.0, 162.0, 137.4, 129.5, 128.7, 128.7, 127.9, 127.0, 79.3, 60.7, 60.3, 55.4, 37.4, 27.7.

MS (MALDI-TOF): *m*/*z* = 443.0 [M + K].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{20}H_{24}DN_3O_6$: 427.1698; found: 427.1697.

(*S*,*E*)-4-(α-D-Diazoacetoxy)but-2-enyl 2-[(*tert*-Butoxycarbonyl)amino]-4-methylpentanoate (32)
Yellow oil; yield: 23.7 mg (0.064 mmol, 88%); ≥95% D-incorporation.

$[\alpha]_D^{21}$ –5.6 (*c* 1.0, $CHCl_3$).

IR (ATR): 2959, 2108, 1686, 1304, 1158 cm^{-1}.

^1H NMR (300 MHz, CD_3CN): δ = 5.82–5.65 (m, 2 H), 5.58 (s, 1 H, NH), 4.99 (residual 1 H), 4.76–4.66 (m, 4 H), 4.11 (t, *J* = 6.5 Hz, 1

H), 1.73–1.59 (m, 1 H), 1.51 (t, *J* = 7.2 Hz, 2 H), 1.40 (s, 9 H), 0.91 (t, *J* = 6.6 Hz, 6 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 173.3, 162.0, 156.0, 128.6, 128.0, 79.1, 60.6, 60.3, 52.5, 40.4, 27.8, 24.8, 22.4, 21.0.

MS (MALDI-TOF): *m*/*z* = 393.2 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{17}H_{26}DN_3NaO_6$: 393.1855; found: 393.1844.

Ethyl (*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-hydroxypropanoate (33)

Synthesised according to a modified procedure;[40] spectral data match those previously reported for this compound.[41]

To a soln of ethyl (*S*)-2-amino-3-hydroxypropanoate hydrochloride (150 mg, 0.9 mmol) in a mixture of 1,4-dioxane (2.5 mL) and H_2O (2.5 mL), $NaHCO_3$ (220 mg, 2.7 mmol) and Boc_2O (0.23 mL, 0.97 mmol) were successively added at 0 °C. The reaction mixture was allowed to warm to r.t. and stirred for 16 h. After that time, the solution was extracted with EtOAc (3 × 5 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford a white solid; yield: 130 mg (0.56 mmol, 62%).

Ethyl (*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(diazoacetoxy)propanoate

Synthesised according to a published procedure.[28] To a soln of ethyl (*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropanoate (**33**; 0.3 g, 1.3 mmol) and K_2CO_3 (0.89 g, 6.4 mmol) in anhyd CH_2Cl_2 (7 mL), was added bromoacetyl bromide (0.34 mL, 3.9 mmol) dropwise via syringe at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 10 min at 0 °C and H_2O (5 mL) was added. The solution was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The thus-obtained residue was dissolved in anhyd THF (7 mL) and *N*,*N*′-ditosylhydrazine (0.88 g, 2.6 mmol) was added. The reaction mixture was cooled to 0 °C and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.96 mL, 6.4 mmol) was added dropwise via syringe under a nitrogen atmosphere. The reaction mixture was stirred for 10 min at 0 °C and sat. aq $NaHCO_3$ (5 mL) was added. The solution was extracted with Et_2O (3 × 10 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. Purification of the product by silica gel chromatography (Et_2O–PE, 1:9) afforded the title product as a yellow oil; yield: 0.09 g (0.30 mmol, 24%).

$[\alpha]_D^{21}$ +42.5 (*c* 1.0, $CHCl_3$).

IR (ATR): 2979, 2113, 1694, 1502, 1391, 1348, 1159, 1057, 1026, 858 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.24 (s, 1 H), 4.70 (s, 1 H), 4.54–4.27 (m, 3 H), 4.15 (q, *J* = 7.2 Hz, 2 H), 1.38 (s, *J* = 7.7 Hz, 9 H), 1.21 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.7, 155.2, 80.2, 64.5, 61.8, 53.1, 46.2, 28.1, 13.9.

MS (MALDI-TOF): *m*/*z* = 324.2 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{12}H_{19}N_3NaO_6$: 324.1166; found: 324.1163.

Ethyl (*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(α-D-diazoacetoxy)propanoate (34)

To a solution of ethyl (*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(2-diazoacetoxy)propanoate (30 mg, 0.1 mmol) and K_2CO_3 (0.14 mg, 0.001 mmol) in MeCN (0.2 mL) was added D_2O (0.18 mL). The resulting mixture was stirred vigorously under nitrogen for 2 h at r.t. After that time, the solution was extracted with CH_2Cl_2 (3 × 2 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford a yellow oil; yield: 25.7 mg (0.085 mmol, 85%); ≥95% D-incorporation.

$[\alpha]_D^{21}$ +43 (*c* 1.0, $CHCl_3$).

IR (ATR): 2980, 2110, 1685, 1310, 1157 cm^{-1}.

^{1}H NMR (300 MHz, CD_3CN): δ = 5.79 (s, 1 H, NH), 4.97 (residual 1 H), 4.46–4.31 (m, 3 H), 4.24–4.08 (m, 2 H), 1.41 (s, 9 H), 1.23 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (100 MHz, CD_3CN): δ = 169.9, 155.6, 79.5, 64.0, 61.7, 53.4, 27.7, 13.7.

MS (MALDI-TOF): *m*/*z* = 326.4 [M + Na].

HRMS (NSI): *m*/*z* [M + Na] calcd for $C_{12}H_{18}DN_3NaO_6$: 325.1229; found: 325.1231.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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