

Cite this: *Chem. Sci.*, 2024, 15, 15399 All publication charges for this article have been paid for by the Royal Society of Chemistry

Gram-scale enzymatic synthesis of 2'-deoxyribonucleoside analogues using nucleoside transglycosylase-2[†]

Admir Salihovic,^{ab} Alex Ascham,^c Andrea Taladriz-Sender,^{ab} Samantha Bryson,^{ab} Jamie M. Withers,^{ab} Iain J. W. McKean,^{ab} Paul A. Hoskisson,^d Gideon Grogan^{bc} and Glenn A. Burley^{ab}

Nucleosides are pervasive building blocks that are found throughout nature and used extensively in medicinal chemistry and biotechnology. However, the preparation of base-modified analogues using conventional synthetic methodology poses challenges in scale-up and purification. In this work, an integrated approach involving structural analysis, screening and reaction optimization, is established to prepare 2'-deoxyribonucleoside analogues catalysed by the type II nucleoside 2'-deoxyribosyltransferase from *Lactobacillus leichmannii* (LINDT-2). Structural analysis in combination with substrate profiling, identified the constraints on pyrimidine and purine acceptor bases by LINDT2. A solvent screen identifies pure water as a suitable solvent for the preparation of high value purine and pyrimidine 2'-deoxyribonucleoside analogues on a gram scale under optimized reaction conditions. This approach provides the basis to establish a convergent, step-efficient chemoenzymatic platform for the preparation of high value 2'-deoxyribonucleosides.

Received 24th July 2024
Accepted 26th August 2024

DOI: 10.1039/d4sc04938a

rsc.li/chemical-science

Introduction

Nucleosides are essential building blocks used throughout all forms of life.¹ In addition, these analogues are used extensively in biotechnology and the pharmaceutical industry, serving as chemical probes when incorporated into oligodeoxyribonucleotides (ODNs) and genomic DNA,^{2–7} as well as anticancer/antiviral therapeutics.^{8–11} Underpinning many aspects of their utility is the need to modify the nucleobase of the nucleoside scaffold (1–4, Fig. 1A).^{12–14} Modifications range from the incorporation of bio-orthogonal reactive handles, modifications to enhance stability of the *N*-glycosidic linkage,^{15,16} and the incorporation of isotopes to aid structural characterization.^{17–20}

The preparation of nucleobase-modified nucleosides typically requires a multi-step synthetic sequence ranging from the elaboration of the existing purine/pyrimidine nucleobase through to more extensive preparation of analogues *via N*-

glycosylation.^{21,22} Many of these synthetic steps to prepare 2'-deoxyribonucleosides by chemical synthesis require a series of protection/deprotection sequences, and in the context of *N*-glycosylation, produce regioisomeric and anomeric mixtures that can be challenging to purify.

Enzymatic synthesis of nucleoside analogues potentially offers a sustainable, cost and step-efficient alternative to purely chemical synthetic approaches. Of the various enzymes used in their biocatalytic synthesis,²³ nucleoside phosphorylases (NPs) have been the most prominent (Fig. 1B).^{24,25} NPs catalyze a 'nucleobase swap' where, typically, the nucleobase of a natural nucleoside feedstock is exchanged for a non-natural nucleobase.^{26,27} Purine NPs show substrate tolerance for a range of purine nucleobases as well as a range of modifications to the ribosugar,^{25,28} whereas pyrimidine NPs can accept modifications to the pyrimidine nucleobase.^{29–31}

A mechanistic hallmark of NPs is that nucleobase exchange proceeds *via* a two-step process involving *N*-glycosidic cleavage of a requisite nucleoside **5** by phosphate, producing pentose-1-phosphate **6** and a nucleobase.³² The formation of **7** then involves nucleophilic displacement of the C1' phosphate in **6** with a nucleobase analogue, exclusively forming the desired β -anomer (**7**).

One major challenge of NP-catalysed nucleobase swapping is that the synthesis of the desired nucleoside product (*e.g.*, **7**) is in equilibrium with the competitive formation of **6**. As such, efforts have focused on developing strategies to mitigate the undesirable reverse reactions by biasing the equilibrium

^aDepartment of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, UK G1 1XL. E-mail: glenn.burley@strath.ac.uk

^bStrathclyde Centre for Molecular Bioscience, University of Strathclyde, UK

^cDepartment of Chemistry, University of York, Heslington, York, YO10 5DD, UK. E-mail: gideon.grogan@york.ac.uk

^dStrathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UK

[†] Electronic supplementary information (ESI) available: The authors have cited additional references within the ESI.^{39,76–100} Metadata of this work is deposited on <https://pure.strath.ac.uk>. See DOI: <https://doi.org/10.1039/d4sc04938a>



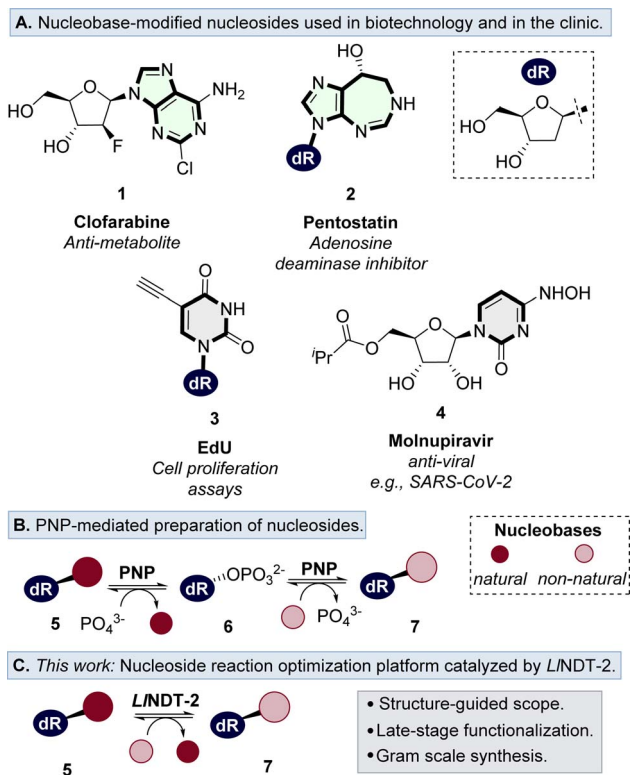


Fig. 1 (A) Examples of prominent nucleoside analogues used in the clinic and in biotechnology. (B) Overview of the utility of NPs as biocatalysts for the preparation of nucleoside analogues. (C) This work: establishing a structure-guided workflow for the optimization of the reaction conditions to prepare nucleoside analogues catalysed by *L*INDT-2.

towards formation of the desired nucleoside analogue,^{28,33} building a feedstock of **6**,³⁴ or through the application of flow-based synthetic routes.^{35–37}

An alternative to the use of NPs to prepare nucleoside analogues is to use nucleoside 2'-deoxyribosyltransferases (NDTs).^{38,39} Whilst these enzymes catalyse the formation of nucleoside analogues *via* an overall nucleobase exchange akin to NPs, NDTs form a covalent adduct within the active site, thus controlling stereoselective nucleophilic attack of the incoming nucleobase on the β face (Fig. 1C). The mechanistic divergence of NDTs offers potential advantages over NPs as they do not form a phosphate intermediate such as **6**.^{40,41}

At present, in-depth knowledge of the substrate promiscuity of NDTs has yet to be established,^{40,42–46} particularly with respect to the ability of these enzymes to be used for scale-up. Herein, we describe a workflow for the gram-scale preparation of nucleoside analogues catalyzed by a type-2 NDT derived from *Lactobacillus leichmannii* (*L*INDT-2).^{47–49} Structural studies highlight potential sites where modification of the nucleobase are tolerated. We use this knowledge to map the substrate promiscuity of both purine and pyrimidine substrates. Finally, we use this knowledge to establish sustainable conditions for the cost-effective preparation of prominent nucleoside analogues.

Results and discussion

Solvent tolerance of transglycosylation catalyzed by *L*INDT-2

The first phase of establishing an optimization workflow was to survey reaction conditions for the synthesis of non-natural nucleosides (*i.e.*, **7**) where the higher value non-natural nucleobase is the limiting reagent. An excess (5 equiv.) of 2'-deoxycytidine (dC, **8**, ~£50 for 250 mg, Merck) was used as the corresponding 2'-deoxyribose donor relative to 1 equiv. of a non-natural purine **9** or pyrimidine **10** (Fig. 2A). Transglycosylation catalysed by *L*INDT-2 showed remarkable tolerance to a range of organic solvents (20% v/v) and even pure water, producing the desired purine **11** or pyrimidine **12** nucleosides in conversions of up to 98% (**11**) and 85% (**12**, Fig. 2B).

Based on the solvent screening we explored the further optimization of the synthesis of a high value pyrimidine nucleoside, **3**. Nucleoside **3** (EdU, ~£160 for 50 mg, Merck) is used extensively in cellular proliferation assays,⁴ as a Raman active reporter,^{50,51} and as a bio-orthogonal reactive group for the synthesis of bioconjugates.⁵² When 5 equiv. of **8** were used as the 2'-deoxyribose donor, the transformation resulted in 53% conversion to **3** using **13** as the nucleobase (entry 1, Fig. 2C). Increasing the enzyme loading from 2 to 4 $\mu\text{g mL}^{-1}$ (from a 1 mg mL⁻¹ stock solution), increased the conversion to a maximum of 66% (entries 2–4). Critical to increasing the conversion of **3** was increasing the equivalents of **13** from 1 to 10 whilst maintaining the catalytic loading of *L*INDT-2 at 4 $\mu\text{g mL}^{-1}$ (entries 5 and 6). A further increase to 20 equiv. of **13** resulted in 90% conversion to **3** (entry 8). The scope of these optimized conditions was used to scale up the synthesis of pyrimidine nucleoside analogues modified at the 5-position (Fig. 2D). 5-Modified nucleosides were then prepared on millimole scale to demonstrate the scalability of this biocatalytic reaction. In this way, **3** and **12** were prepared on 1 mmol scale in 52% (132 mg) and 55% (137 mg) yields, respectively. Preparation of **14** and **15** was also demonstrated affording the desired products in 56% (840 mg) and 31% (53 mg), respectively.

Structural basis for superior acceptance of purine nucleobases

A consistent observation that emerged from the solvent screen was that higher conversions resulted when purine **9** was used as the nucleobase 'acceptor' relative to pyrimidine **10**. Although previous studies have reported lower transglycosylation conversions when pyrimidine nucleobases were used as acceptors,^{42,53,54} a structural basis for this has not been explored. We surmised that the generally lower conversions to non-natural pyrimidine nucleoside products is due to the reduced residence time of the smaller pyrimidine nucleobase relative to a larger purine analogue in the active site of *L*INDT-2, which in turn could lead to competing hydrolysis.^{38,54–57} Indeed, a recent study of the reaction kinetics of a related NDT derived from *Chroococcidiopsis thermalis* (*Ct*INDT) revealed preference for purine nucleobase donors, which was largely driven by the K_M .⁵⁸

To shed further light on the substrate scope of *L*INDT-2, we sought to obtain structures of the enzyme in complex with 2'-



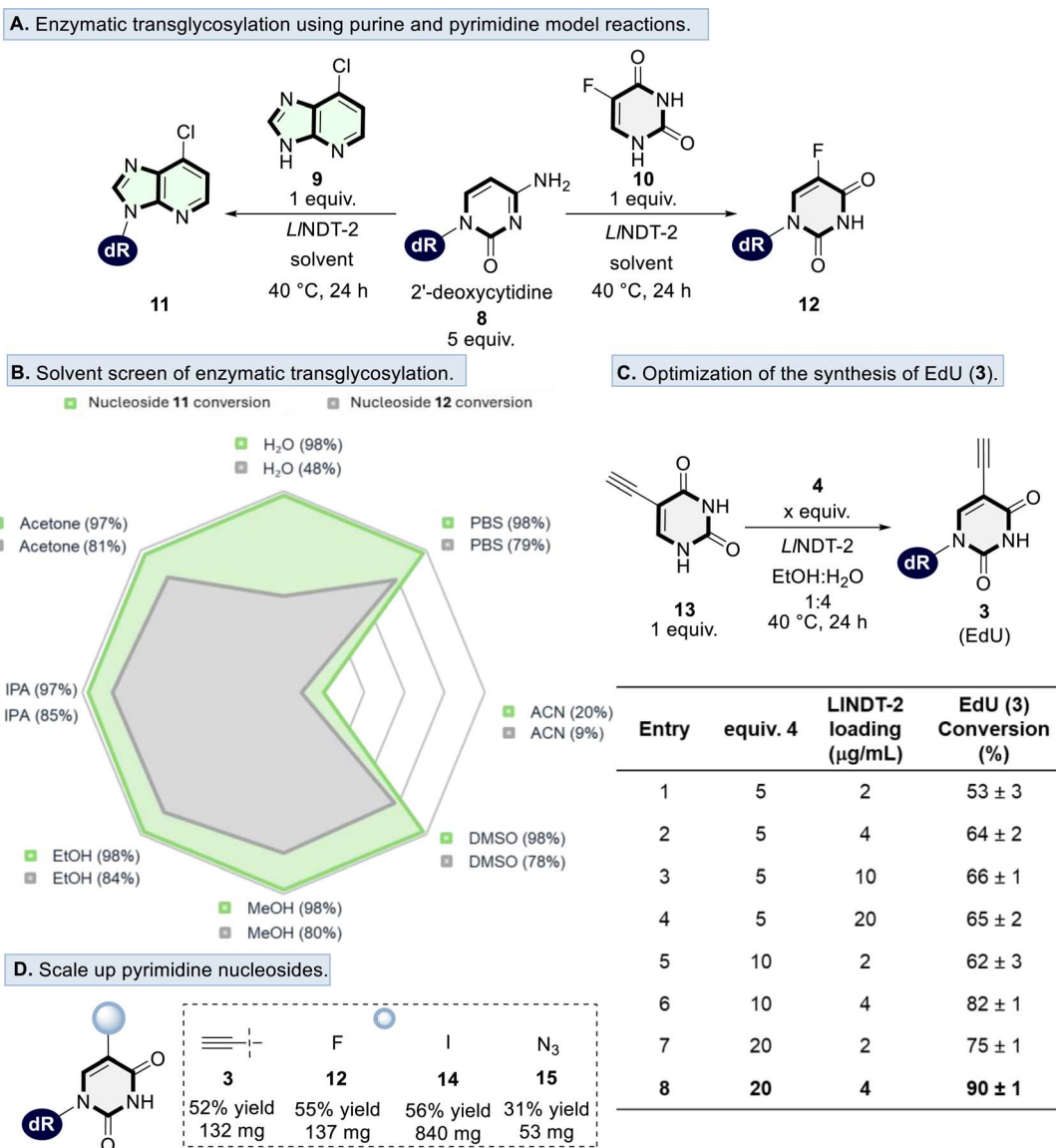


Fig. 2 (A) Overview of the model reactions used to screen solvent dependency of transglycosylation catalysed by *LINDT-2*. (B) % conversion to nucleoside products **11** and **12** as a function of solvent. The % conversion was calculated by the ratio of the peak area of nucleobase to the peak area of the nucleoside product. (C) Optimization of the conversion of **3** (EdU) by surveying the number of equivalents of 2'-deoxyribsyl donor and *LINDT-2*. General reaction conditions: **4** (5–20 equiv.), nucleobase (1 equiv.), solvent (EtOH : H₂O, 1 : 4), *LINDT-2* (2 to 20 μg mL⁻¹). (D) Scale up of pyrimidine nucleoside analogues. Reaction conditions: **4** (3–10 equiv.), nucleobase (1 equiv.), solvent (H₂O), *LINDT-2* (2 to 4 μg mL⁻¹), r.t. or 40 °C, 24 h.

deoxyribonucleosides. The structure of WT-*LINDT-2* (EC 2.4.2.6) in complex with the *C*-nucleoside 5-methyl-2'-deoxypseudouridine (PDB: 1F8Y), which cannot undergo transglycosylation, was previously determined by Ealick and co-workers.³⁹ Although that report also featured details of a complex of *LINDT* with 2'-deoxyadenosine, no coordinate file for this complex is available in the PDB, so a reconsideration of complex interactions is timely.

Three datasets were obtained from crystals prepared under different substrate soak conditions. Each structure was obtained in the *I*₂3 space group and featured two monomers of *LINDT-2* in the asymmetric unit. We first obtained an apo structure of *LINDT-2*, which had not been soaked with substrate

ligands, and featured no ligand density in the active sites. The second structure was obtained from crystals soaked with **3** (EdU). Both active sites featured density between the side chains of E98 and D92 in a 'closed' conformation of the enzyme corresponding to that observed previously (Fig. 3A).³⁹ This density was modelled as the covalent 2'-deoxyribsyl enzyme complex **16**, evidenced by continuous density from the side chain of E98 to the C1' atom of the deoxyribose sugar. Although crystallographic evidence for this intermediate in DRTase-I Class enzymes has previously been reported,⁵⁹ the structure of a covalent 2'-deoxyribsyl adduct (Fig. 3B) has not been observed in a Class II enzyme. Additional density adjacent to the C-terminus of Y157 was not sufficiently large to model as a base.



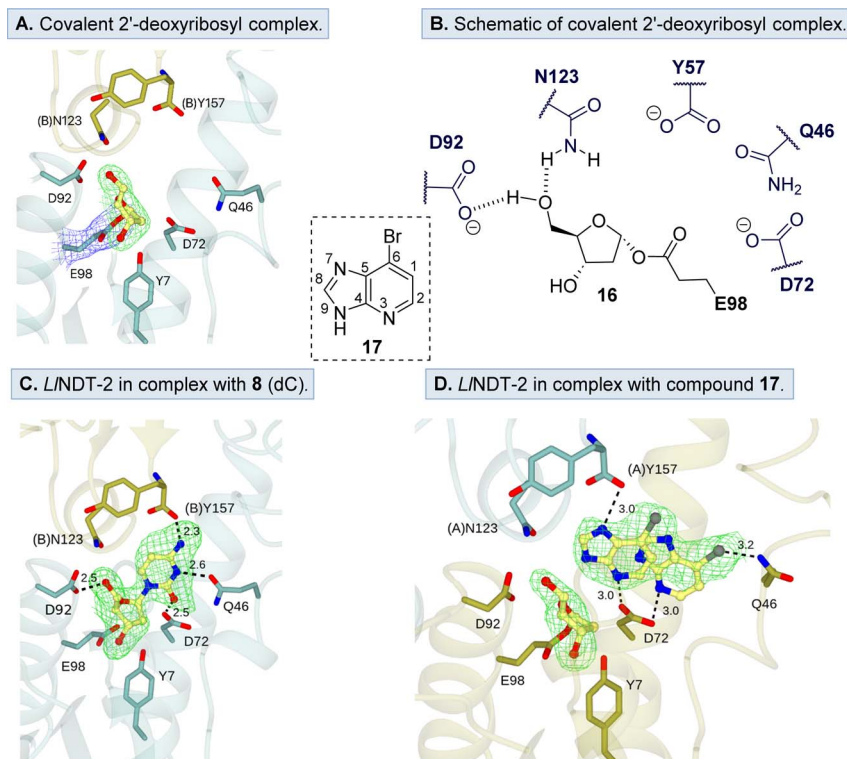


Fig. 3 (A) Formation of a covalent *LINDT-2* complex containing the 2'-deoxyribosyl intermediate in the active site (from Dataset #2). (B) Schematic depiction of the covalent complex **16** and the nucleobase **17**. (C) *LINDT-2* in complex with **8** (dC). 'A' monomer from Dataset #3. (D) *LINDT-2* in complex with 2'-deoxyribosyl intermediate and **16**. 'B' monomer from Dataset #3. Monomers 'A' and 'B' are presented with carbon atoms in blue and gold, respectively. Electron density in blue and green corresponds to the $2F_o - F_c$ and $F_o - F_c$ (omit) maps at levels of 1σ and 3σ . The maps were obtained before the refinement of the ligand atoms in each case. Selected interactions are illustrated by black dashed lines with distances in Å.

A third structure was obtained by soaking the enzyme with nucleoside **50** (Fig. 3C and 5A). This structure contained one active site in the closed conformation containing **8** (dC). The non-covalent interactions with active site residues in this complex largely align with the previously reported 5-methyl-2'-deoxyuridine complex (PDB: 1F8Y).³⁹ The exocyclic amine of **8** forms a non-covalent interaction with the C-terminus of Y157, whereas the endocyclic N3 forms a hydrogen bond with the side chain of Q46. Finally, hydrogen bond interactions were observed between O2 of **8** and both Q46 and D72 side chains.

The other enzyme monomer was observed in an 'open' conformation with the side chain of Q46 displaced because of a movement of the protein backbone between residues L43 and Y58. This active site featured electron density consistent with the covalent 2'-deoxyribosyl intermediate attached *via* an ester linkage from E98, and also two molecules of the nucleobase **17** in overlapping locations in a purine binding site (Fig. 3D). The purine interactions within the active site side chains are similar to those previously reported in the paper by Ealick *et al.*, however no coordinates are available in the PDB for a thorough comparison.³⁹ For the molecule of **17** situated closest to the 2'-deoxyribosyl adduct, N9 is 3.9 Å from the C1' of the sugar, to which it would be bonded in an intact nucleoside; the N7 atom is 3.0 Å from the C-terminus of Y157, and N3 is 3.0 Å from the side-chain of D72. Another molecule of **17** is also present in the

active site and is accommodated by movement of the Q46 side chain, forming a non-covalent interaction with the bromine atom at C6.

Taken collectively, our structural analyses suggest that the residence time of purine nucleobases within the active site of *LINDT-2* is greater than that observed for pyrimidine nucleobases. This is consistent with previous reaction kinetics analysis of *LINDT-2* where the equilibrium is biased towards complexation of a purine nucleobase within the active site.^{38,54}

Substrate mapping defines the scope of the transglycosylation catalysed by *LINDT-2*

With the structural information in hand, we then sought to construct a detailed molecular map defining the substrate scope of non-natural nucleoside synthesis using the optimised biotransformation conditions as a basis (Fig. 4). Using either **8** (dC) or thymidine as the 2'-deoxyribosyl donor, the scope of nucleobase acceptance was explored using a range of pyrimidine analogues modified at the 5-position (**18–22**, Fig. 4). Our structures of *LINDT-2* suggest that the active site can accommodate substituents at this position as they project into a space between the side chains of Y157, F13 and N123. Unexpectedly, the lack of substituents in the 4-position (**23**) and nucleobase modifications to the 2- (**26–27**), 3- (**24**) and 4-positions (**25–27**) were also tolerated. However, an acceptor nucleobase with



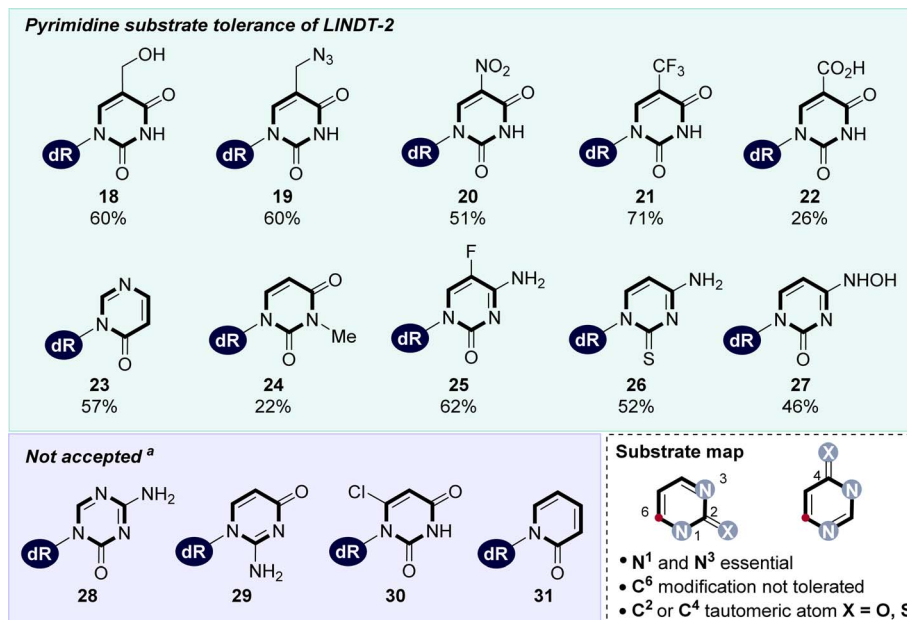


Fig. 4 Substrate scope of transglycosylation using pyrimidine nucleobases. General reaction conditions: **8** (dC) or thymidine (5–10 equiv.), nucleobase (1 equiv.), solvent (organic : H₂O, 1 : 4), *L*INDT-2 (2 to 4 μg mL⁻¹), 40 °C, 24 h. ^aNot accepted correlates to <10% conversion.

a substituent at position 6 (**30**) was not tolerated as this may clash with the side chain of N123. Nucleobases such as triazine (**28**),⁶⁰ hydrogen bond donor groups in the 2-position (**29**) or pyridine (**31**) were not substrates, confirming that the N3 is essential.⁶¹ This is presumably due to the need for the nucleophilic nitrogen to be adjacent to a tautomeric oxygen.⁶¹

The acceptance of purine nucleobase analogues by *L*INDT-2 was far more extensive. Purines with modifications at all carbon positions were tolerated, producing non-natural nucleosides **32–51** with moderate to high conversions (Fig. 5A). Purine acceptors with a range of modifications at both C2 and C6 positions (**32–40**) were accepted, including precursors for the preparation of 6-thioguanosine (**36**), a known DNA damage adduct *O*⁶-methyl guanosine **37**, and those including protecting groups used for solid phase DNA synthesis (e.g., **38**).

The active site of *L*INDT-2 in complex with the nucleobase **17** revealed that the mobility of the loop bearing Q46 permits the accommodation of a range of modifications at the C2 and C6 positions (e.g., **41–44**).

Substrate mapping also identified tolerance for modifications at the N1-position. For example, transglycosylation using 1-deaza acceptors were tolerated (**45–47**) as well as modifications at the C8 (**48–50**) and C6 positions (**51**). Surprisingly, a trifluoromethyl substituent in the C8 position forms nucleoside **49**, where N3 is the glycosylated position instead of N9.⁶² Another unexpected observation was the low conversion in the synthesis of 8-aza nucleoside **50**, which was formed with 11% conversion. Nitrogen atoms at both N3 and N7 positions were essential for acceptor recognition, as the corresponding deazapurine analogues were not transformed to the desired nucleoside products (**52–56**). This is presumably due to the removal of

specific interactions with the C-terminal carboxylate of Y157 and the side chain of D72 respectively.

Having established the broader substrate specificity of *L*INDT, we were able to scale up several syntheses of purine nucleoside analogues using our optimised reactions conditions (Fig. 5C). In this way, nucleosides **32**, **36**, **42**, **45** and **51** were prepared on 0.6–6.5 mmol scale, affording products ranging from 90 mg to 1.37 g. Finally, to demonstrate the wider applicability of this enzymatic transglycosylation approach for the preparation of high value nucleoside building blocks, we sought to prepare the ¹⁵N-labelled nucleoside **58** starting from **51** (Fig. 5C). ¹⁵N-labelled nucleosides are used extensively in structural biology and as chemical probes to explore their enzymatic incorporation into DNA.^{19,63–66} The incorporation of ¹⁵N building blocks bearing non-natural nucleobases typically involve the incorporation of the ¹⁵N label, followed by a glycosylation step. Glycosylation is achieved enzymatically using a NP,⁶⁷ or *via* a synthetic glycosylation step (e.g., Vorbrüggen)^{68,69} to prepare the final nucleoside.⁶⁵ Accordingly, we prepared the novel ¹⁵N-labelled nucleoside **58** in two steps starting from **51**. Acetyl protection of the hydroxyl groups afforded **57** in 90% yield, which was necessary to solubilise the nucleoside for the Pd-catalysed cross coupling with ¹⁵N-labelled benzoyl amide. The cross coupling and acetyl deprotection were performed in one-pot on a 0.3 mmol scale, affording **58** in 45% yield (48 mg).

Conclusions

The promiscuity of *L*INDT-2-catalysed transglycosylations using non-natural nucleobases has been demonstrated using either 2'-deoxycytidine (**8**) or thymidine as the corresponding 2'-deoxyribose donor. Structural analysis of *L*INDT-2 provided insight into the determinants of acceptor specificity by *L*INDT,



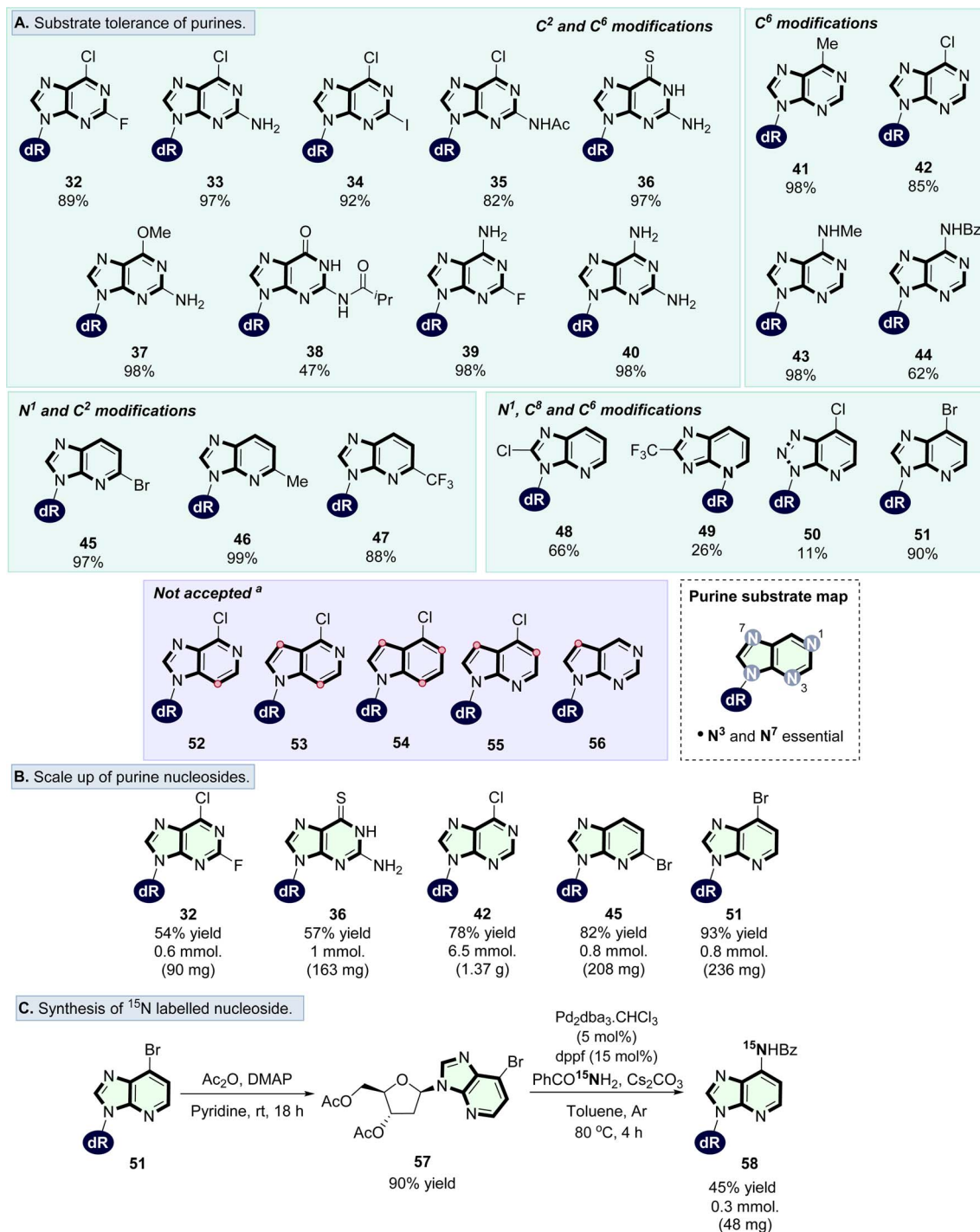


Fig. 5 (A) Substrate scope of transglycosylation using purine nucleobases. General reaction conditions: **8** (dC) or thymidine (5–10 equiv.), nucleobase (1 equiv.), solvent (organic : H₂O, 1 : 4), *L*INDT-2 (0.2% v/v to 0.4% v/v, 2 to 4 μ L), 40 °C, 24 h. (B) Scale up representative purine nucleosides. (C) Application of *L*INDT-2-mediated transglycosylation for the synthesis of the ¹⁵N labelled nucleoside **53**. ^aNot accepted is <10% conversion.

and thus the generally lower conversion to pyrimidine 2'-deoxyribonucleosides compared with purine analogues using equivalent stoichiometry. These transglycosylation reactions are inherently scalable, thus providing an alternative to the use of NPs, which can suffer from the need to prepare a pentose-1-phosphate (**6**) to drive the equilibrium to completion.^{70,71} This

work highlights a workflow to prepare a wider range of nucleoside analogues with sugar modifications typically found in, for example, therapeutic oligonucleotides and nucleoside drugs.^{72–74} Future work will explore the potential to use engineered *L*INDT-2 enzymes to enhance substrate scope for



acceptors with modifications at the 2'-position^{44,75} and the subsequent potential to prepare nucleosides on scale.^{35,53}

Abbreviations

L/NDT- Type II nucleoside 2'-deoxyribosyltransferase from
2 *Lactobacillus leichmannii*
NP Nucleoside phosphorylase

Data availability

The preparation of compounds and associated raw spectroscopic and HPLC data to confirm identity and purity will be made available through the University of Strathclyde's Pure repository (<https://pure.strath.ac.uk>). Protein crystallographic data has been uploaded to the PDB.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Conceptualization was done by A. S., A. T. S., P. A. H., and G. A. B.; methodology was performed by A. S., A. A., S. B., J. M. W., I. J. W. M., G. G.; formal analysis was done by A. S., A. A. and G. G. Manuscript preparation was done by A. S., G. G. and G. A. B.

Conflicts of interest

The authors declare no competing financial conflict of interest.

Acknowledgements

A. S. and G. A. B. thank the University of Strathclyde and the EPSRC for a PhD studentship. A. A. was funded by a studentship from the BBSRC White Rose DTP in Mechanistic Biology (ref. 2737877). A. T. S. and G. A. B. thank the Biotechnology and Biological Research Council (BBSRC) for support (BB/V017586/1; BB/T000627/1). J. W. and G. A. B. thank the Leverhulme Trust for funding support (RPG-2018-149). P. A. H. acknowledges funding from the Royal Academy of Engineering Research Chair Scheme (RCSRF2021\11\15). A. S. and G. A. B. thank the MVL Integrated Protein Analysis Facility at the University of Glasgow, for their guidance and advice with protein purification. We also thank Mr Sam Hart and Dr Johan P. Turkenburg for assistance with X-ray data collection, and the Diamond Light Source Didcot UK for access to beamline I03 under proposal mx32736.

References

1 Y. Wang, S. Zhang, W. Jia, P. Fan, L. Wang, X. Li, J. Chen, Z. Cao, X. Du, Y. Liu, K. Wang, C. Hu, J. Zhang, J. Hu, P. Zhang, H.-Y. Chen and S. Huang, *Nat. Nanotechnol.*, 2022, **17**, 976–983.

- X. Tang, J. Zhang, J. Sun, Y. Wang, J. Wu and L. Zhang, *Org. Biomol. Chem.*, 2013, **11**, 7814–7824.
- J. Kuchlyan, L. Martinez-Fernandez, M. Mori, K. Gavvala, S. Ciaco, C. Boudier, L. Richert, P. Didier, Y. Tor, R. Improta and Y. Mély, *J. Am. Chem. Soc.*, 2020, **142**, 16999–17014.
- A. Salic and T. J. Mitchison, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 2415–2420.
- G. A. Burley, J. Gierlich, M. R. Mofid, H. Nir, S. Tal, Y. Eichen and T. Carell, *J. Am. Chem. Soc.*, 2006, **128**, 1398–1399.
- V. N. Schreier, M. O. Loehr, E. Lattmann and N. W. Luedtke, *ACS Chem. Biol.*, 2022, **17**, 1799–1810.
- N. Z. Fantoni, A. H. El-Sagheer and T. Brown, *Chem. Rev.*, 2021, **121**, 7122–7154.
- S. Westarp, F. Kaspar, P. Neubauer and A. Kurreck, *Curr. Opin. Biotechnol.*, 2022, **78**, 102829.
- G. Li, T. Yue, P. Zhang, W. Gu, L.-J. Gao and L. Tan, *Molecules*, 2021, **26**, 923.
- D. C. Schultz, R. M. Johnson, K. Ayyanathan, J. Miller, K. Whig, B. Kamalia, M. Dittmar, S. Weston, H. L. Hammond, C. Dillen, J. Ardanuy, L. Taylor, J. S. Lee, M. Li, E. Lee, C. Shoffler, C. Petucci, S. Constant, M. Ferrer, C. A. Thaiss, M. B. Frieman and S. Cherry, *Nature*, 2022, **604**, 134–140.
- C. M. Galmarini, J. R. Mackey and C. Dumontet, *Lancet Oncol.*, 2002, **3**, 415–424.
- A. Hottin and A. Marx, *Acc. Chem. Res.*, 2016, **49**, 418–427.
- A. Omumi, D. G. Beach, M. Baker, W. Gabryelski and R. A. Manderville, *J. Am. Chem. Soc.*, 2011, **133**, 42–50.
- M. Hocek and M. Fojta, *Chem. Soc. Rev.*, 2011, **40**, 5802–5814.
- J. Štambaský, M. Hocek and P. Kočovský, *Chem. Rev.*, 2009, **109**, 6729–6764.
- D. F. Vargas, E. L. Larghi and T. S. Kaufman, *ACS Omega*, 2021, **6**, 19356–19363.
- L. T. Olenginski, K. M. Taiwo, R. M. LeBlanc and T. K. Dayie, *Molecules*, 2021, **26**, 5581.
- K. Lu, Y.-C. Hsiao, C.-W. Liu, R. Schoeny, R. Gentry and T. B. Starr, *Chem. Res. Toxicol.*, 2022, **35**, 7–29.
- F. H. T. Nelissen, M. Tessari, S. S. Wijmenga and H. A. Heus, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2016, **96**, 89–108.
- F. X. Theillet, *Chem. Rev.*, 2022, **122**, 9497–9570.
- E. K. Davison, D. A. Petrone, M. Meanwell, M. B. Nodwell, S. M. Silverman, L.-C. Campeau and R. Britton, *Nat. Protoc.*, 2022, **17**, 2008–2024.
- C. Maria and A. P. Rauter, *Carbohydr. Res.*, 2023, **532**, 108889.
- S. C. Cosgrove and G. J. Miller, *Expert Opin. Drug Discovery*, 2022, **17**, 355–364.
- S. Kamel, I. Thiele, P. Neubauer and A. Wagner, *Biochim. Biophys. Acta, Proteins Proteomics*, 2020, **1868**, 140304.
- A. Hatano, H. Wakana, N. Terado, A. Kojima, C. Nishioka, Y. Iizuka, T. Imaizumi and S. Uehara, *Catal. Sci. Technol.*, 2019, **9**, 5122–5129.
- M. D. Erion, J. D. Stoeckler, W. C. Guida and R. L. Walter, *Biochemistry*, 1997, **36**, 11735–11748.



- 27 M. J. Pugmire and S. E. Ealick, *Structure*, 1998, **6**, 1467–1479.
- 28 F. Kaspar, M. Seeger, S. Westarp, C. Köllmann, A. P. Lehmann, P. Pausch, S. Kemper, P. Neubauer, G. Bange, A. Schallmeyer, D. B. Werz and A. Kurreck, *ACS Catal.*, 2021, **11**, 10830–10835.
- 29 K. Szeker, X. Zhou, T. Schwab, A. Casanueva, D. Cowan, I. A. Mikhailopulo and P. Neubauer, *J. Mol. Catal. B: Enzym.*, 2012, **84**, 27–34.
- 30 X. Zhou, K. Szeker, B. Janocha, T. Boehme, D. Albrecht, I. A. Mikhailopulo and P. Neubauer, *FEBS J.*, 2013, **280**, 1475–1490.
- 31 F. Kaspar, P. Neubauer and A. Kurreck, *ChemBioChem*, 2021, **22**, 1385–1390.
- 32 T. H. Tahirov, E. Inagaki, N. Ohshima, T. Kitao, C. Kuroishi, Y. Ukita, K. Takio, M. Kobayashi, S. Kuramitsu, S. Yokoyama and M. Miyano, *J. Mol. Biol.*, 2004, **337**, 1149–1160.
- 33 F. Kaspar, R. T. Giessmann, K. F. Hellendahl, P. Neubauer, A. Wagner and M. Gimpel, *ChemBioChem*, 2020, **21**, 1428–1432.
- 34 M. A. Huffman, A. Fryszkowska, O. Alvizo, M. Borra-Garske, K. R. Campos, K. A. Canada, P. N. Devine, D. Duan, J. H. Forstater, S. T. Grosser, H. M. Halsey, G. J. Hughes, J. Jo, L. A. Joyce, J. N. Kolev, J. Liang, K. M. Maloney, B. F. Mann, N. M. Marshall, M. McLaughlin, J. C. Moore, G. S. Murphy, C. C. Nawrat, J. Nazor, S. Novick, N. R. Patel, A. Rodriguez-Granillo, S. A. Robaire, E. C. Sherer, M. D. Truppo, A. M. Whittaker, D. Verma, L. Xiao, Y. Xu and H. Yang, *Science*, 2019, **366**, 1255–1259.
- 35 A. I. Benitez-Mateos and F. Paradisi, *ChemSusChem*, 2022, **15**, e202102030.
- 36 A. I. Benitez-Mateos, C. Klein, D. Roura Padrosa and F. Paradisi, *Catal. Sci. Technol.*, 2022, **12**, 6231–6238.
- 37 X. Zhou, K. Szeker, L.-Y. Jiao, M. Oestreich, I. A. Mikhailopulo and P. Neubauer, *Adv. Synth. Catal.*, 2015, **357**, 1237–1244.
- 38 S. A. Short, S. R. Armstrong, S. E. Ealick and D. J. T. Porter, *J. Biol. Chem.*, 1996, **271**, 4978–4987.
- 39 S. Armstrong, W. J. Cook, S. A. Short and S. E. Ealick, *Structure*, 1996, **4**, 97–107.
- 40 J. Del Arco, J. Acosta and J. Fernández-Lucas, *Biotechnol. Adv.*, 2021, **51**, 107701.
- 41 J. del Arco, A. Perona, L. González, J. Fernández-Lucas, F. Gago and P. A. Sánchez-Murcia, *Org. Biomol. Chem.*, 2019, **17**, 7891–7899.
- 42 E. Pérez, P. A. Sánchez-Murcia, J. Jordaan, M. D. Blanco, J. M. Mancheño, F. Gago and J. Fernández-Lucas, *ChemCatChem*, 2018, **10**, 4406–4416.
- 43 J. Li, L. Yu, J. Li, L. Xie, R. Zhang and H. Wang, *J. Biosci. Bioeng.*, 2019, **128**, 22–27.
- 44 Y.-J. Yoo, K.-H. Choi, B.-K. Kim, S.-S. Choi and E.-S. Kim, *J. Microbiol. Biotechnol.*, 2022, **32**, 1041–1046.
- 45 J. Acosta, J. Del Arco, V. Pisabarro, F. Gago and J. Fernández-Lucas, *Front. Bioeng. Biotechnol.*, 2020, **8**, 593.
- 46 A. Fresco-Taboada, I. de la Mata, M. Arroyo and J. Fernández-Lucas, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 3773–3785.
- 47 S. Vichier-Guerre, T. C. Ku, S. Pochet and K. L. Seley-Radtke, *ChemBioChem*, 2020, **21**, 1412–1417.
- 48 S. Vichier-Guerre, L. Dugue, F. Bonhomme and S. Pochet, *Org. Biomol. Chem.*, 2016, **14**, 3638–3653.
- 49 S. Pochet, L. Dugué, A. Meier and P. Marlière, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1679–1684.
- 50 S. Hong, T. Chen, Y. Zhu, A. Li, Y. Huang and X. Chen, *Angew. Chem., Int. Ed.*, 2014, **53**, 5827–5831.
- 51 H. Yamakoshi, K. Dodo, A. Palonpon, J. Ando, K. Fujita, S. Kawata and M. Sodeoka, *J. Am. Chem. Soc.*, 2012, **134**, 20681–20689.
- 52 J. Gierlich, K. Gutschiedl, P. M. E. Gramlich, A. Schmidt, G. A. Burley and T. Carell, *Chem.–Eur. J.*, 2007, **13**, 9486–9494.
- 53 F. Rinaldi, J. Fernández-Lucas, D. de la Fuente, C. Zheng, T. Bavaro, B. Peters, G. Massolini, F. Annunziata, P. Conti, I. de la Mata, M. Terreni and E. Calleri, *Bioresour. Technol.*, 2020, **307**, 123258.
- 54 D. J. T. Porter and S. A. Short, *J. Biol. Chem.*, 1995, **270**, 15557–15562.
- 55 J. Fernández-Lucas, L. A. Condezo, F. Martinez-Lagos and J. V. Sinisterra, *Enzyme Microb. Technol.*, 2007, **40**, 1147–1155.
- 56 M. Smar, S. A. Short and R. Wolfenden, *Biochemistry*, 1991, **30**, 7908–7912.
- 57 J. Acosta, E. Pérez, P. A. Sánchez-Murcia, C. Fillat and J. Fernández-Lucas, *Biomolecules*, 2021, **11**, 120.
- 58 P. Tang, C. J. Harding, A. L. Dickson, R. G. da Silva, D. J. Harrison and C. M. Czekster, *ACS Catal.*, 2024, **14**, 3090–3102.
- 59 R. Anand, P. A. Kaminski and S. E. Ealick, *Biochemistry*, 2004, **43**, 2384–2393.
- 60 M. A. Konkina, M. S. Drenichev, D. I. Nasyrova, Y. B. Porozov and C. S. Alexeev, *Sustainable Chem. Pharm.*, 2023, **32**, 101011.
- 61 R. Cardinaud and J. Holguin, *Biochim. Biophys. Acta*, 1979, **568**, 339–347.
- 62 M.-C. Huang, J. A. Montgomery, M. C. Thorpe, E. L. Stewart, J. A. Secrist and R. L. Blakley, *Arch. Biochem. Biophys.*, 1983, **222**, 133–144.
- 63 G. Kupferschmitt, J. Schmidt, T. Schmidt, B. Fera, F. Buck and H. Riiterjans, *Nucleic Acids Res.*, 1987, **15**, 6225–6241.
- 64 M. Terrazas, X. Ariza, J. Farràs, J. M. Guisado-Yang and J. Villarrasa, *J. Org. Chem.*, 2004, **69**, 5473–5475.
- 65 I. M. Lagoja and P. Herdewijn, *Synthesis*, 2002, 301–314.
- 66 F. R. Traube, S. Schiffers, K. Iwan, S. Kellner, F. Spada, M. Müller and T. Carell, *Nat. Protoc.*, 2019, **14**, 283–312.
- 67 J.-L. Abad, B. L. Gaffney and R. A. Jones, *J. Org. Chem.*, 1999, **64**, 6575–6582.
- 68 T. K. Dayie, L. T. Olenginski and K. M. Taiwo, *Chem. Rev.*, 2022, **122**, 9357–9394.
- 69 J. P. Henschke, X. Zhang, X. Huang, L. Mei, G. Chu, K. Hu, Q. Wang, G. Zhu, M. Wu, C. Kuo and Y. Chen, *Org. Process Res. Dev.*, 2013, **17**, 1419–1429.



- 70 F. Kaspar, F. Brandt, S. Westarp, L. Eilert, S. Kemper, A. Kurreck, P. Neubauer, C. R. Jacob and A. Schallmey, *Angew. Chem., Int. Ed.*, 2023, **62**, e202218492.
- 71 F. Kaspar, R. T. Giessmann, P. Neubauer, A. Wagner and M. Gimpel, *Adv. Synth. Catal.*, 2020, **362**, 867–876.
- 72 K. J. D. Van Giesen, M. J. Thompson, Q. Meng and S. L. Lovelock, *JACS Au*, 2023, **3**, 13–24.
- 73 A. J. Burke, W. R. Birmingham, Y. Zhuo, T. W. Thorpe, B. Z. da Costa, R. Crawshaw, I. Rowles, J. D. Finnigan, C. Young, G. M. Holgate, M. P. Muldowney, S. J. Charnock, S. L. Lovelock, N. J. Turner and A. P. Green, *J. Am. Chem. Soc.*, 2022, **144**, 3761–3765.
- 74 E. R. Moody, R. Obexer, F. Nickl, R. Spiess and S. L. Lovelock, *Science*, 2023, **380**, 1150–1154.
- 75 M. Willmott, W. Finnigan, W. R. Birmingham, R. Heath, S. Derrington, C. Schnepel, M. A. Hayes, P. D. Smith, F. Falcioni and N. J. Turner, *ChemRxiv*, 2023, preprint, DOI: [10.26434/chemrxiv-2023-26t6z](https://doi.org/10.26434/chemrxiv-2023-26t6z).
- 76 T. Wenzel and F. Seela, *Helv. Chim. Acta*, 1996, **79**, 169–178.
- 77 S. Dey, C. L. Rühl and A. Jäschke, *Chem.–Eur. J.*, 2017, **23**, 12162–12170.
- 78 F. Yang, Y. Zhu and B. Yu, *Chem. Commun.*, 2012, **48**, 7097–7099.
- 79 T. S. Rao, R. F. Rando, J. H. Huffman and G. R. Revankar, *Nucleosides Nucleotides*, 1995, **14**, 1997–2008.
- 80 W. Kabsch, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2010, **66**, 133–144.
- 81 P. Evans, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2006, **62**, 72–82.
- 82 G. Winter, *J. Appl. Crystallogr.*, 2010, **43**, 186–190.
- 83 P. Emsley and K. Cowtan, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2004, **60**, 2126–2132.
- 84 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1997, **53**, 240–255.
- 85 X. Xu, S. Yan, J. Hu, P. Guo, L. Wei, X. Weng and X. Zhou, *Tetrahedron*, 2013, **69**, 9870–9874.
- 86 S. Fernández-García, V. O. Chantzakou and F. Juliá-Hernández, *Angew. Chem., Int. Ed.*, 2024, **63**, e202311984.
- 87 D. Tauraitė, J. Jakubovska, J. Dabužinskaitė, M. Bratchikov and R. Meškys, *Molecules*, 2017, **22**, 672.
- 88 K. Kraszewska, I. Kaczyńska, S. Jankowski, J. Karolak-Wojciechowska and E. Sochacka, *Bioorg. Med. Chem.*, 2011, **19**, 2443–2449.
- 89 X.-Y. Jin, Y.-M. He, T.-H. Hui, L. Liu and L. Cheng, *J. Org. Chem.*, 2024, **89**, 3597–3604.
- 90 M. J. Robins, M. MacCoss, S. R. Naik and G. Ramani, *J. Am. Chem. Soc.*, 1976, **98**, 7381–7389.
- 91 T. Suzuki, A. Ozawa-Tamura, M. Takeuchi and Y. Sasabe, *Chem. Pharm. Bull.*, 2021, **69**, 1067–1074.
- 92 C. H. Hwang, J. S. Park, J. H. Won, J. N. Kim and E. K. Ryu, *Arch. Pharmacol. Res.*, 1992, **15**, 69–72.
- 93 J. von Watzdorf and A. Marx, *ChemBioChem*, 2016, **17**, 1532–1540.
- 94 Z. Janeba, X. Lin and M. J. Robins, *Nucleosides, Nucleotides Nucleic Acids*, 2004, **23**, 137–147.
- 95 R. Xia, L.-S. Chen, S.-H. Xu, C. Xia and L.-P. Sun, *Nucleosides, Nucleotides Nucleic Acids*, 2022, **41**, 593–604.
- 96 P. Leonczak, P. Srivastava, O. Bande, G. Schepers, E. Lescrinier and P. Herdewijn, *J. Org. Chem.*, 2019, **84**, 13394–13409.
- 97 M. J. Uddin, M. I. Schulte, L. Maddukuri, J. Harp and L. J. Marnett, *Nucleosides, Nucleotides Nucleic Acids*, 2010, **29**, 831–840.
- 98 H. Challa and T. C. Bruice, *Bioorg. Med. Chem.*, 2004, **12**, 1475–1481.
- 99 H. Yehia, S. Westarp, V. Röhrs, F. Kaspar, R. T. Giessmann, H. F. T. Klare, K. Paulick, P. Neubauer, J. Kurreck and A. Wagner, *Molecules*, 2020, **25**, 934.
- 100 F. Seela, Y. Chen, U. Bindig and Z. Kazimierzczuk, *Helv. Chim. Acta*, 1994, **77**, 194–202.

