Contactless digital tags are increasingly penetrating into many areas of human activities. Digitalization of our environment requires an ever growing number of objects to be identified and tracked with machine-readable labels. Molecules offer immense potential to serve for this purpose, but our ability to write, read, and communicate molecular code with current technology remains limited. Here we show that magnetic patterns can be synthetically encoded into stable molecular scaffolds with paramagnetic lanthanide ions to write digital code into molecules and their mixtures. Owing to the directional character of magnetic susceptibility tensors, each sequence of lanthanides built into one molecule produces a unique magnetic outcome. Multiplexing of the encoded molecules provides a high number of codes that grows double-exponentially with the number of available paramagnetic ions. The codes are readable by nuclear magnetic resonance in the radiofrequency (RF) spectrum, analogously to the macroscopic technology of RF identification. A prototype molecular system capable of 16-bit (65,535 codes) encoding is presented. Future optimized systems can conceivably provide 64-bit (~10^19 codes) or higher encoding to cover the labelling needs in drug discovery, anti-counterfeiting and other areas.
Managing digital information is crucial not only for our civilization but for the existence of life itself. All known life forms depend on nucleic acids, which are in essence digital medium. The information capacity of molecules is enormous and very tempting for technological applications. Indeed, humans learned to hack the system of nucleic acids for the purpose of encoding data. However, the principle of molecular recognition that works well within a biological microenvironment is difficult to connect with technology built primarily around the electromagnetism of inorganic materials and RF communication. Thus, alternative synthetic polymers have been proposed for information encoding. Unfortunately, the current methods of reading often result in the destruction of the molecules. In contrast, modern civilization demands to communicate information wirelessly and repeatably, in an effort to identify and track objects, monitor health, and eventually merge the digital and the biological scales.

Peptides can offer the desired modular synthetic flexibility at the molecular level can be achieved with either (i) sequences of monomers concatenated within one molecular string or (ii) mixtures of individual, unique compounds. For both, there is a trade-off between synthetic difficulty, material demands, and information capacity. Long sequences can be made from a few monomers, but synthetic writing is difficult, slow, and prone to errors. On the other hand, mixtures are easy to make but require a high number of uniquely distinguishable components, which eventually become a limiting factor. Theoretically, an alternative approach, better balancing the need to generate many codes with a small number of components and synthetic steps, is (iii) to first synthesize short sequences and then use them as unique components in mixtures. Although this approach is conceptually well-understood, the difficulty of decoding mixed sequences is prohibitive.

Reading (decoding) nongenomic molecular codes currently heavily relies on mass spectrometry (MS), which can decode sequences as well as complex mixtures with high sensitivity. However, mixtures of sequences containing permutations of the same monomers have the same molecular weight and are nearly impossible to decode. In addition, MS is a destructive method that does not allow for repeated readings that are desirable for long-term tracking and monitoring. On the other hand, spectroscopic methods which are capable of this can typically only detect individual components (monomers) but cannot distinguish their order in sequences. Another challenge for spectroscopic methods is signal overlaps that limit the number of distinguishable codes. For example, fluorescent labels, as one of the most important tools for interrogation of biological systems, provide relatively broad signals within a narrow spectral window. Up to ten different fluorescent tags can be distinguished from mixtures with advanced computational methods, but this still amounts only to $2^{10} - 1 = 1023$ codes. This limitation cannot be overcome without expanding the size along at least one dimension. Indeed, various microscale particles have been proposed as barcodes, based on tuneable luminescent materials combined to generate the code. However, the information capacity per mass is much lower for particles compared to molecules. A potential way out of this dilemma is to use non-optical methods of reading. For example, the spectral window of nuclear magnetic resonance (NMR) spectroscopy can accommodate much higher number of distinguishable signals usable for information encoding.

In the same way that magnetic media accelerated modern information technologies, it is recognized that molecular magnetism could be a major breakthrough for future technologies at the molecular scale. Alas, single-molecule magnets so far function only at impractically low temperatures. The next best magnetic alternative that works at the molecular level and over a broad temperature range simultaneously is paramagnetism. When placed within an external magnetic field, unpaired electrons of a paramagnetic metal ion generate their own local field described by a magnetic susceptibility tensor. This tensor has specific radial and angular dependencies dictated by the particular element and its coordination environment, and strongly influences the NMR frequencies of nearby nuclei, an effect exploited in protein structure elucidation. Lanthanides are ideally suited for this purpose, as they provide a range of chemically similar but magnetically different ions amenable to stable incorporation into organic molecules via coordination. While distinguishable NMR signals can be obtained from chemically different molecules, decoupling of the NMR shifts from chemical properties. This principle has been previously used to create chemically nearly identical molecules that could be spectrally resolved with magnetic resonance imaging (MRI). However, with one lanthanide ion per molecule, the number of distinguishable signals remains limited to 12 usable lanthanides (excluding Pm, Gd; including 1 diamagnetic). So far, only a few works have investigated the possibility of combining paramagnetic effects of multiple lanthanides within one molecule, presumably due to the difficulty in synthesizing well-defined multimetallic compounds. We speculated that two or more lanthanide ions arranged in sequence within one molecule should combine their magnetic susceptibility tensors to produce a unique NMR-readable outcome for each permutation of the elements. This greatly increases the number of signals and molecular codes that can be generated with a limited number of elements (Fig. 1).

In this work, we present a prototype of paramagnetically encodeable molecular architecture, demonstrate the encoding and decoding principles, provide practical examples of information encoding, and outline the possible future capabilities. The number of unique codes that can be generated in this way from a few chemical building blocks is compatible with the foreseeable needs in drug discovery and anti-counterfeiting applications. This work provides an alternative direction toward programmable digital molecular information systems.

Results

Design of building blocks. Combining magnetic susceptibility tensors of lanthanide ions requires their placement in close proximity within one molecule, preferably in a modular way to enable encoding of specific sequences. Conformational freedom and isomerism must be limited, so that the metal ion positions are well defined and there is no averaging or multiplication of NMR signals. Peptides can offer the desired modular synthetic approach, provided that a suitable building block is available to act simultaneously as amino acid and a strong chelator for Ln$^{3+}$ ions. Several amino acid/chelator building blocks have been described previously, but none were suitable for the task, falling short of the required control over conformations and/or isomerism. Learning from these examples, we developed a family of building blocks collectively named DO3A-Hyp, where the chelator is based on macroyclic DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) and the amino acid is derived from hydroxyproline (Hyp). Here, we present two members of the family, building blocks \( L_1 \) and \( L_2 \) (Fig. 2 and synthetic scheme in Fig. 3), that differ in the configuration of chiral centers in the Hyp moiety. The DO3A-Hyp family is structurally related to the chelator HP-DO3A that is clinically used in the MRI contrast agent Gadoteridol. Although knowing
A sequence of two lanthanide ions (M1M2) is chemically written into a tripeptide molecule M1M2-TP1, which incorporates two stereochemically well-defined amino acid/chelator building blocks. The paramagnetic effects of the lanthanides combine to influence a reporter CF3 group. Each M1M2 permutation of the elements (codon) results in a unique 19F NMR shift (δ) that is given by the sum of individual paramagnetic shift contributions from each M1 and M2 (δ1 and δ2, respectively) and a constant diamagnetic shift (δ3). The encoded molecules are further multiplexed to produce codes readable by NMR or MRI. A small number of elements allows the writing of a very high number of codes. The exact chemical structures of the amino acid/chelator building blocks used in this work are provided in Figs. 2 and 3.

Encodable molecular framework. Having the suitable building blocks, we designed a prototype of an encodable molecular framework. Tripeptide 1 (TP1) consists of two L1 units, one at each terminus, and a middle amino acid bearing a reporter CF3 group. Analogous tripeptide 2 (TP2) molecule contains L2 unit at the C-terminus. This construction allows the reporter to perceive magnetic fields from two metal ions simultaneously in M1M2-TP1 or M1M2-TP2 molecules (Fig. 5). We selected the CF3 reporter for the high sensitivity and negligible natural background of the 19F nucleus, which facilitate observation and interpretation of signals in the RF NMR channel. The chemical shifts induced by paramagnetic lanthanide ions are difficult to predict from theory with confidence, especially if the structure in solution is not precisely known. Therefore, to

The exact structure of the chelates was not necessary for the purpose of paramagnetic encoding, we found with X-ray diffraction on a [Dy(L2)] crystal that inversion (2S→2R,3S,4S) occurred in Hyp during synthesis of L1 (Fig. 4A and Supplementary Figs. 1–3). We explain this by deprotonation of the epoxide 5 during its reaction with basic cyclen (suggested mechanism in Supplementary Fig. 4). The same reaction step also opened a path to the isomer L2, though with a much lower yield (9:1 ratio L1: L2). The crystal structure of [Dy(L2)] confirmed 2S,3R,4S configuration of Hyp in L2 (Fig. 4B and Supplementary Figs. 5–7). In both solid-state structures, the Hyp moiety coordinated with the 2R- or 2S-carboxyl, respectively, rather than with the 4S-hydroxyl group. Nevertheless, the 2R-carboxyl of [Ln(L1)] chelates was reactive in peptide coupling reactions (in contrast to unreactive coordinated acetate arms), indicating coordination competition with 4S-hydroxyl in solution (Supplementary Fig. 8). Coordination of 4S-hydroxyl is therefore considered in figures to respect this reactivity. Our assumption was that the chiral Hyp moiety would impose a preference for one of several possible coordination isomers typical for macrocyclic chelators and thus manage the problem of isomeric speciation. Indeed, only one dominant species was found with NMR spectroscopy in solution for all tested lanthanides (see the next section).
gain a quick overview, we used a metal-free TP1 for post-
synthetic complexation of equimolar binary lanthanide mixtures
to obtain statistical mixtures of four $M^1M^2$-TP1 compounds in
1:1:1:1 molar ratio (Fig. 5B). In all studied cases, $^{19}$F NMR spectra
showed four major singlets, mostly free of overlaps, shifted and
broadened by the paramagnetic action of the respective $Ln^{3+}$ ions
(Supplementary Fig. 9). We selected the $Dy^{3+}/Ho^{3+}$ combination
for further detailed study because of a good balance between
chemical shift and NMR signal broadening (Fig. 6A). Specific

M$^1$M$^2$ sequences were encoded into $M^1M^2$-TP1 compounds via
controlled synthesis using several different strategies, some of
which also included direct peptide coupling with metal-preloaded
derivatives of L$^1$ (Supplementary Figs. 64–86). Each of the well-
defined $M^1M^2$-TP1 compounds matched one of the four major
$^{19}$F NMR peaks observed in the statistical $Dy^{3+}/Ho^{3+}$ mixture,
confirming that each $M^1M^2$ sequence generates a unique signal
(Fig. 6B). Some minor peaks detected in the $^{19}$F NMR spectra of
(purified) $M^1M^2$-TP1 compounds could be attributed to low-

Fig. 3 Synthetic scheme for building blocks L$^1$, L$^2$, and their protected variants. Conditions: (i) BnBr (benzyl bromide), TEA (triethylamine), THF
(tetrahydrofuran); (ii) PPh$_3$, THF, 0 °C followed by DIAD (diisopropyl azodicarboxylate), Mel, 0 °C → RT; (iii) (PhSe)$_2$, EtOH, NaBH$_4$, 0 °C; (iv) H$_2$O$_2$, THF, 0 °C → RT; (v) MCPBA (meta-chloroperoxybenzoic acid), CHCl$_3$, 85 °C; (vi) cyclen, t-BuOH, 105 °C; (vii) t-BuO$_2$CC$_2$Br, K$_2$CO$_3$, MeCN; (viii) H$_2$, Pd@C, AcOH, MeOH; (ix) TFA; (x) FmocCl, aq. borate/NaOH buffer (pH 9.0), MeCN; (xi) MeO$_2$CCH$_2$Br, K$_2$CO$_3$, MeCN; (xii) Ac$_2$O, TEA, DMAP (4-
dimethylaminopyridine), MeCN. Intermediates in brackets were not isolated. Colors de-

Fig. 4 Molecular structures of [Dy(L$^1$)] and [Dy(L$^2$)] in the solid state. In both structures, the ligand coordinated to the central Dy$^{3+}$ cation with four ring
nitrogen donors and four carboxylate donors. The view highlights the absolute configuration of the proline ring. Thermal ellipsoids were set at 50% probability.
A [Dy(L$^1$)] contains $2R,3S,4S$ configuration of the proline ring (green shading) and the chelate adopts Δλλλλ square antiprismatic (SA)
conformation. For detailed information, see Supplementary Figs. 1-3. B [Dy(L$^2$)] contains $2S,3R,4S$ configuration of the proline ring (violet shading) and the
chelate adopts Δ6666 twisted square antiprismatic (TSA) conformation. For detailed information, see Supplementary Figs. 5-7.
abundance forms of the same compound (conformers or isomers), because most of them coalesced with the main peak at elevated temperatures (Supplementary Fig. 10). Nevertheless, these minor peaks presented no obstacle in further analysis of the NMR data. As expected, the induced chemical shifts strongly depended also on the precise coordination environment and mutual arrangement of the two metal centers. The molecular system of M1M2-TP2, despite its (deceptive) similarity to M1M2-TP1, provided dramatically different chemical shifts with the same metal ion sequences (Fig. 6C and Supplementary Fig. 11). Furthermore, to investigate the importance of rigid and compact molecular design on paramagnetic encoding, we performed a control experiment using an alternative architecture. The same Dy3+/Ho3+ sequences were encoded into a tripeptide TP3 that was constructed with the previously described DOTA-K building block49,50 containing a flexible sidechain linker (Supplementary Fig. 12). Conformational flexibility, in this case, resulted in metal ion position averaging, diminishing the paramagnetic shifts, and consequently the TP3 system failed to produce distinguishable signals for different M1M2 sequences (Fig. 6D and Supplementary Fig. 13). Moreover, the signals appeared split, likely due to the cis–trans isomerism of the coordinated amide moiety previously noted for similar compounds36. This problem was not observed with the TP1 and TP2 systems using the DO3A-Hyp family building blocks, demonstrating that a rigid and stereochemically well-defined molecular framework is essential for the purpose of paramagnetic encoding.

**Rules of paramagnetic encoding.** Various molecular systems have been previously explored that used a single lanthanide ion per molecule to generate discernible NMR shifts, primarily for imaging applications43–45. However, the way that magnetic susceptibility tensors of multiple lanthanide ions combine within one molecule is a rarely studied phenomenon46,47. We, therefore, examined the rules of this in the M3M3-TP1 system in detail. Our hypothesis was that the observed 19F chemical shift (\(\delta_F\)) was a sum of three independent parameters:

\[
\delta_F = \delta_{p1} + \delta_{p2} + \delta_d,
\]

where \(\delta_{p1}\) and \(\delta_{p2}\) are individual pseudocontact paramagnetic shifts generated by metal M in a given position (1 or 2, as defined in Fig. 1), and \(\delta_d\) is a constant diamagnetic contribution.

To test this hypothesis, we first analyzed a limited dataset comprised of Dy3+/Ho3+, Dy3+/Y3+ and Ho3+/Y3+ statistical mixtures of M3M3-TP1 compounds. The diamagnetic Y3+ ion was included to anchor the \(\delta_d\) component. The assignment of peaks to specific SM1M2 sequences was already partially known (Fig. 6B) and the rest was inferred or determined by trial and error. A multivariate least-square fit according to Eq. (1) converged to a single set of \(\delta_{p1}, \delta_{p2}, \delta_d\) values. An independent dataset of Dy3+/Sm3+, Dy3+/Lu3+, Sm3+/Lu3+ mixtures provided similar \(\delta_{p1}, \delta_{p2}\) values for Dy3+ that was present in both datasets and nearly identical \(\delta_d\) (Supplementary Fig. 14). These results demonstrated that the contributions of individual paramagnetic centers were independent and additive, confirming the hypothesis. Knowing the parameters for Dy3+, Ho3+, and Sm3+ allowed us to decipher \(\delta_{p1}, \delta_{p2}\) values for the remaining tested lanthanides. Final global fit provided very good agreement between the calculated and measured shifts, with residual differences not exceeding 0.34 ppm within a 38.8 ppm overall range (Supplementary Fig. 15). Because paramagnetic shifts are very sensitive to changes in molecular structure, this agreement demonstrates that the M3M3-TP1 compounds were practically isostructural regardless of the inserted lanthanide ions. For each element, the \(\delta_{p1}, \delta_{p2}\) values were of opposite signs (Supplementary Fig. 15B), indicating substantial mutual rotation of the two magnetic susceptibility tensors in M3M3-TP1 molecule35. The \(\delta_{p1}\) and \(\delta_{p2}\) values roughly followed tabulated Bleaney constants35.

Fig. 5 Synthesis of M1M2-TP1 and M1M2-TP2 compounds. A Main synthetic pathway to well-defined M1M2-TP1 products. B Post-synthetic complexation pathway to statistical mixtures of four major M1M2-TP1 products. C Synthetic pathway to well-defined M1M2-TP2 products (analogous to A). Conditions: (i) Fmoc-Phe(S-CF3)-OH, PyAOP (7-azabenzo[2,1,5]tetrazolo[4,3-b]pyridine-13-yl)tritylpyrrolidinophosphonium hexafluorophosphate), DIPEA (N,N-diisopropylethylamine), DMF (dimethyl sulfoxide) followed by DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), DMF (dimethylformamide); (ii) Ac-Fmoc-Me3L1, PyAOP, DIPEA, DMSO followed by TFA (trifluoroacetic acid); (iii) M3Cl2, aq. MOPS (3-(N-morpholino)propanesulfonic acid)/NaOH (pH 7.0) followed by LiOH, H2O, MeOH; (iv) M3Cl2 aq. MOPS/NaOH (pH 7.0); (v) LiOH, H2O, MeOH; (vi) M3Cl2, M3Cl2, aq. MOPS/NaOH (pH 7.0). For the full structures of the building blocks, see Fig. 3. Detailed synthetic procedures including alternative synthetic pathways are provided in Supplementary Figs. 17–95.
thus showing a reasonable agreement with theory (Supplementary Fig. 16), although the accuracy of Bleaney’s theoretical model has recently been questioned \cite{35}. Limited synthetic yields prevented us from performing a similar extensive analysis on the M\textsuperscript{1}M\textsuperscript{2}-TP2 system, but from the comparison in Fig. 6, it is clear that the \(\delta_p\) values must be very different from M\textsuperscript{1}M\textsuperscript{2}-TP1.

**Multiplexing and combinatorial implications.** Multiplexing is a way to encode information by combining unique signals into composite patterns that can be received through a single channel and decoded back to the original components. Chemical compounds are usable for this purpose by virtue of their signals being detectable by analytical methods. For example,

**Sequence decoding.** Current strategies to decode sequences of nongenomic oligomers mostly rely on their sequential degradations (e.g., Edman degradation) or fragmentation with tandem MS techniques \cite{8,9,10}, resulting in the destruction of the molecules. Non-destructive spectroscopic methods typically cannot read sequences of monomer units. This is different for paramagnetic metal ions, where the magnetic susceptibility tensors bring directionality. Metal ion sequences in the M\textsuperscript{1}M\textsuperscript{2}-TP1 system can be decoded from one-dimensional NMR spectra, provided that the \(\delta_p\) components are known and the \(\delta_d\) shifts are uniquely distinguishable. The latter condition is somewhat limiting, but, nevertheless, suitable sets of elements can be identified. We found by simulation that Tb\textsuperscript{3+}, Dy\textsuperscript{3+}, Ho\textsuperscript{3+}, and Yb\textsuperscript{3+} can be freely combined within the M\textsuperscript{1}M\textsuperscript{2}-TP1 system, with a minimum peak distance of 0.36 ppm (Supplementary Fig. 17). Moreover, a particularly important property of paramagnetic encoding is that the basic parameters of the system (i.e., \(\delta_p\), \(\delta_d\) values in the case of M\textsuperscript{1}M\textsuperscript{2}-TP1) can be obtained from limited experimental data and then used to reliably simulate and identify sequences that were not yet encountered. This is significant for the design of advanced future systems, where the number of combinations may be too high to be comprehensively explored by experimentation. We tested the reliability of the predictions and sequence decoding on the sub-system of M\textsuperscript{1}M\textsuperscript{2}-TP1 compounds encoded with Tb\textsuperscript{3+}/Dy\textsuperscript{3+}/Ho\textsuperscript{3+}/Yb\textsuperscript{3+} ions. To ensure that we erased prior knowledge, we excluded from the experimental data all cases where these elements were combined in statistical mixtures. An unbiased set of \(\delta_d\) values was fitted from this reduced dataset (Supplementary Fig. 18). Using these parameters, we simulated shifts for all 16 permutations. The best match between the experimental \(\delta_d\) and simulated \(\delta_d\) shifts confidently identified all 12 heteronuclear M\textsuperscript{1}M\textsuperscript{2} sequences (Supplementary Fig. 19), none of which was used to obtain the \(\delta_d\) values that allowed prediction of the \(\delta_d\).
Digital files have been encoded into mixtures of organic compounds readable by mass spectrometry\textsuperscript{19,20} or fluorescence spectroscopy\textsuperscript{23}. Lanthanides were previously multiplexed to generate molecular barcodes based on their unique isotopic mass\textsuperscript{33}, luminescence\textsuperscript{24}, or paramagnetic NMR shifts\textsuperscript{45}. However, decoding of such chemical systems typically proceeds with Boolean logic, considering only the presence/absence of each component, and ignoring their repetition and order. This severely limits the number of codes that can be generated, especially when the number of basic components itself is limited (e.g., chemical elements). For example, two elements can provide only three unique codes readable by luminescence or mass (Fig. 7A). Notably, this applies also to multiplexing NMR signals of molecules that carry a single lanthanide ion\textsuperscript{45}. Paramagnetic encoding based on multiple lanthanides within each molecule improves the combinatorics by inserting permutations into the process. First, two elements combine into a codon, where repetitions and order are recognized, resulting in 4 permutations with unique signals. In the second step, these codons are multiplexed, providing 15 unique codes (Fig. 7B). A comprehensive physical realization of this two-level encoding is demonstrated in Fig. 7C with the $\text{M}^1\text{M}^2\text{TP1}$ system and $\text{Dy}^{3+}/\text{Ho}^{3+}$ ions. Controlled synthesis was essential for the whole encoding process to work. If uncontrolled post-synthetic 4 elements and considering codon size 2. Lanthanides were previously multiplexed to generate molecular barcodes based on their unique isotopic mass\textsuperscript{33}, luminescence\textsuperscript{24}, or paramagnetic NMR shifts\textsuperscript{45}. However, decoding of such chemical systems typically proceeds with Boolean logic, considering only the presence/absence of each component, and ignoring their repetition and order. This severely limits the number of codes that can be generated, especially when the number of basic components itself is limited (e.g., chemical elements). For example, two elements can provide only three unique codes readable by luminescence or mass (Fig. 7A). Notably, this applies also to multiplexing NMR signals of molecules that carry a single lanthanide ion\textsuperscript{45}. Paramagnetic encoding based on multiple lanthanides within each molecule improves the combinatorics by inserting permutations into the process. First, two elements combine into a codon, where repetitions and order are recognized, resulting in 4 permutations with unique signals. In the second step, these codons are multiplexed, providing 15 unique codes (Fig. 7B). A comprehensive physical realization of this two-level encoding is demonstrated in Fig. 7C with the $\text{M}^1\text{M}^2\text{TP1}$ system and $\text{Dy}^{3+}/\text{Ho}^{3+}$ ions. Controlled synthesis was essential for the whole encoding process to work. If uncontrolled post-synthetic
Digital information encoding and reading. To demonstrate a practical example of information encoding, we employed the system of $\text{M}_1\text{M}_2\text{-TP1}$ and $\text{Yb}^{3+}\text{/Ho}^{3+}$ ions. All 16 possible compounds were individually synthesized and provided unique and distinguishable NMR signals (Supplementary Fig. 20). With each signal representing one bit ($\text{present} = 1$, $\text{absent} = 0$), it is possible to encode 16 bits of digital data by multiplexing the compounds. Standard ASCII (American Standard Code for Information Interchange) characters use 7 bits, so a single mixture can carry two characters. That is not enough for practical purposes. Therefore, we organized five different mixtures within a capillary into layers that were spatially separated by an immiscible signal-free solvent $\text{CCl}_4$ (Fig. 8A). Thus, a composite NMR sample was created with the capacity to store ten ASCII characters, enough to encode a reasonably strong password. To read back the information, we used $\text{z}$-resolved NMR spectroscopy to acquire spectra of all layers simultaneously (Fig. 8B). After referencing the chemical shifts in each layer to an internal standard (trifluoroacetic acid, TFA), the presence/absence of the signals was evaluated (Fig. 8C) and converted to a binary code (Fig. 8D). Due to the lower spectral resolution of $\text{z}$-resolved NMR, we avoided the confusion of the two least-resolved peaks ($\text{Dy}^{3+}\text{/Yb}^{3+}$, Fig. 8C) by shifting the beginning of the binary code to one of them ($\text{Dy}^{3+}$), thus eliminating it by definition (binary codes of all standard ASCII characters start with 0, Fig. 8E). Conversion of the binary code to characters revealed the encoded 10-character password (Fig. 8F, G). It is important to note that multiple pairs of positional isomers (e.g., $\text{Yb}^{3+}\text{/Ho}^{3+}$ present in the mixtures make the encoded information practically unreadable with methods other than magnetic resonance since the isomers are very difficult to distinguish by other means. This is a very attractive feature especially for potential counterfeit applications of such molecular codes.

Parallel reading with MRI. A key aspect of reading and communicating information is whether it proceeds in a sequential or parallel way. Parallel systems offer higher and more scalable throughput, which is also suitable for imaging applications. For example, optical reading methods capture information simultaneously from many objects in the field of view, while MS can only process one sample at a time. Magnetic resonance can provide both spectroscopic and spatial information simultaneously, thus allowing parallel reading and imaging. Spectrally resolved MRI has been previously realized with various systems based on chemically different diamagnetic compounds$^{38-41}$, host-guest interactions$^{42,43}$ and lanthanide-induced NMR shifts$^{43-45}$. The latter approach offers the advantage to select the NMR shifts without changing the chemical properties, which is particularly useful for in-vivo imaging applications$^{43,44}$. However, previous systems based on a single lanthanide per molecule offered only a very limited choice. The programmable $\text{M}_1\text{M}_2\text{-TP1}$ system provides an advanced level of control to generate a higher number of practically usable NMR signals. To demonstrate this, we used just two lanthanide ions $\text{Dy}^{3+}/\text{Ho}^{3+}$ to encode the $\text{M}_1\text{M}_2\text{-TP1}$ molecules, which were then pipetted into a $7 \times 5$-well plate in patterns to write one letter with each compound. Each well thus contained a homogenous mixture of 0–4 compounds (Fig. 9A). The whole well plate was imaged on a preclinical 4.7 T MRI scanner with a $^{19}$F CSI (Chemical Shift Imaging) pulse sequence, where two dimensions represent space coordinates and the third is a frequency domain. The information contained in the 35 wells was obtained simultaneously, the encoded molecules were distinguished owing to their different resonance frequencies (Fig. 9B), and the four letters could be resolved and independently displayed (Fig. 9C–F). A fly-through view of the MRI data is provided in Supplementary Movie 1.

Limit of detection. NMR spectroscopy is typically not considered as a method for reading molecular codes because of its relatively low sensitivity compared to e.g., mass spectrometry. This prompted us to investigate the limit of detection achievable with $\text{M}_1\text{M}_2\text{-TP1}$ compounds encoded with $\text{Dy}^{3+}/\text{Ho}^{3+}$ ions. Previous works have demonstrated that shortening of longitudinal relaxation times ($\text{T}_1$) induced by paramagnetic ions allows to use a rapid scan rate and boost sensitivity by acquiring more signal per time$^{33,34}$. We took advantage of this effect in $\text{M}_1\text{M}_2\text{-TP1}$ compounds (Supplementary Fig. 21). The signal-to-noise ratio (SNR) in NMR grows with the square root of the number of scans, but there are practical limits to the experiment time. We extrapolated the detection limit at 11.7 T and SNR = 3 to be 5.8 nmol with a short measurement time (1.6 s, 32 scans), or 123 pmol with a reasonably long measurement time (60 min, 72,000 scans). Paramagnetic codes are therefore conceivably usable as identification tags in bead-based biosamples (a single 90-μm Tentagel bead can hold 100 pmol). The detection limit could be further lowered by technical as well as chemical means, e.g., by optimization of the NMR hardware and pulse sequence, optimizing the molecular system to provide narrower NMR signals (higher SNR), or including a higher number of magnetically equivalent $^{19}$F nuclei. Although the conventional NMR/MRI technology is large and expensive, in principle it can be redesigned for the purpose of reading paramagnetic molecular codes. Down sizing using cost-efficient permanent magnets can dramatically lower the cost and potentially improve the sensitivity to match a particular application$^{36,47}$.

Discussion

Properties of paramagnetically encoded molecules depart from typical chemical systems and approach macroscopic hardware and software. As the encoded information is hidden in chemically inaccessible f-electrons of lanthanides, the hardware (molecule) remains physically unchanged (isostructural) when loaded with different software (metal ion sequence). Simple and clear programming rules, which can be calibrated from a small number of synthesized examples, provide predictable behavior of the molecular code. Reading in the RF spectrum draws parallels with the technology of radiofrequency identification (RFID) tags by being repeatable and non-destructive, and by working through space without a direct line of sight. The paramagnetic codes presented here are nearly chemically identical, based on elements with negligible biological background (lanthanides, fluorine), and structurally related to clinically approved MRI contrast agents. These are favorable qualities for tagging biological objects or artificial objects in the biological milieu. We refer to the $\text{M}_1\text{M}_2\text{-TP1}$ and $\text{M}_1\text{M}_2\text{-TP2}$ systems as prototypes, because many aspects of their modular construction can be further optimized to build more advanced systems with enhanced functionality. The major roadblock to immediate practical use is the low sensitivity of reading with conventional NMR technology. However, rapid advancements in quantum sensors may soon make the detection of single molecules a routine reality$^{98,99}$, opening the door to as-yet unforeseen applications.

Methods

Synthesis of building blocks, tripeptides, and their metal chelates. Detailed synthetic strategies and procedures, including the conditions for the separation of isomers and characterization of all products, are provided in Supplementary Information. All syntheses proceeded in solution. Some lanthanide ions were excluded.
Fig. 8 Password decoding from a single sample with z-resolved NMR. A Physical realization of a single NMR sample containing 5 different mixtures of M1M2-TP1 compounds encoded with Tb3+/Dy3+/Ho3+/Yb3+ ions. Aqueous solutions (in MOPS/NaOH buffer, pH = 7) of the mixtures (layers 1-5) are separated by layers of CCl4. The capillary is submerged in D2O. B Z-resolved 19F NMR spectrum (470 MHz, T = 298.1 K) of the sample from panel A shows signals of the M1M2-TP1 compounds and internal standard (TFA, trifluoroacetic acid) resolved for the layers along the z-axis. Shifts from different layers are not perfectly aligned due to bulk magnetic susceptibility (BMS) effects that varied for each layer. C Evaluation of the signals from panel B by comparison with an independently obtained 1D NMR spectrum of a mixture containing all 16 M1M2-TP1 compounds. Note that peaks of DyDy and YbTb are close but distinguished in the 1D spectrum. Black dots mark shifts from the z-resolved spectrum after correcting for BMS effects in each layer (referencing to TFA). Good match is achieved between expected shifts (vertical lines) and determined shifts (black dots). D Binary representation of the information from C. E Final table of the binary codes for 10 ASCII (American Standard Code for Information Interchange) characters encoded in the sample. Relative to D, the binary codes are rearranged to start at the position of DyDy (green shading), which is not used to avoid confusion with YbTb. F ASCII characters obtained by conversion from the binary codes in E with indication of their order in a password. G The final decoded 10-character password.

from testing for various reasons (La3+: redundant diamagnetic, Gd3+: 3+/4+ redox chemistry, Prn3+: radioactive, Gd3+: inducing no paramagnetic shift). Concentrations of Ln3+ ions in stock solutions for synthesis and in solutions of M1M2-TP1, M1M2-TP2, and M1M2-TP3 compounds were determined with ICP-AES.

X-ray crystallography. Detailed procedures for preparation of the single crystals of [Dy(L)3]:3H2O and [Dy(L)2]:3H2O, details of structure solving and refinement, as well as additional details of the obtained structures are provided in Supplementary Information (Supplementary Methods and Supplementary Figs. 1–3, 5–7, 65, 97).

19F NMR spectroscopy. Measurements of all 19F NMR spectra were done on Avance III HD 500 MHz spectrometer (Bruker, 470.4 MHz for 19F) equipped with a broad-band cryo-probe with ATM module (5 mm CPBBO BB-1H/19F/15N/D 298.1 K) of the sample from panel A – resolved 19F NMR spectrum (470 MHz, T = 298.1 K) of the mixtures (layers 1–7, 57, 97).
This was possible due to the differences in well plate (Image intensities were mapped to colors as shown in the legends. See also Supplementary Movie 1 for a per slice. plate shows four distinct peaks of the four encoded compounds (marked with triangles, color-coding as explained in A). The spectrum was created by projecting the maximum signal intensity from each slice of (CSI) MRI data onto the frequency dimension. The spectral resolution was 19.2 Hz (0.10 ppm) per slice. C-F Selective display of HoDy-TP1 (C), DyDy-TP1 (D), HoHo-TP1 (E) and DyHo-TP1 (F) compounds reveals the patterns encoded with them into the well plate as legible letters (average of 15 adjacent slices for each compound peak). Displayed field of view: 50×50 mm, voxel size 0.78 × 0.78 × 10 mm. Image intensities were mapped to colors as shown in the legends. See also Supplementary Movie 1 for a fly-through view of the MRI data.

GRD). Solutions of samples were prepared in MOPS/NaOH buffer (pH = 7) and placed into an insert capillary that was inserted into a 5-mm NMR tube containing D₂O (used for NMR frequency lock). The volume of solution in the insert observatory by the spectrometer probe was ∼50 μL. Longitudinal relaxation times (T₁) were obtained by inversion recovery sequence. Spectra of statistical mixtures or individually synthesized M₁M₂-TP1 and M₁M₂-TP2 compounds were measured with these parameters: 90°/180° excitation pulse, pre-acquisition delay = 50 ms, receiver gain maximum value = 203. The SNR values were obtained using MestReNovo software (version 12.0.3) after the application of an exponential window function of 1 Hz. The limit of detection (SNR = 3) was extrapolated from SNR measured with 32 scans (1.6 total time), considering that SNR scales linearly with the analyte concentration and with the square root of the number of scans. Further details for SNR measurements are provided in Supplementary Fig. 21. ¹⁹F z-resolved NMR spectrum was acquired using a phase encoding pulse sequence with resolution 2048 × 64 points at 20 mm field of view, number of scans = 32 K, acquisition time = 18 ms, relaxation delay = 100 μs (for technical reasons). The excitation pulse was ∼31°, which was the result of optimization aiming to enhance the SNR of weaker (broader) signals, sacrificing the SNR of sharper peaks. This was possible due to the differences in T₁ relaxation times. The total experimental time was ∼12 h. For subsequent processing, only 700 × 64 raw points were used in order to reduce the noise. Data were apodized using QSIINE window functions in both F2 (SSB = 10) and F1 (SSB = 1) dimensions and zero-filled to 8192 × 128 points. Processing was done in Bruker TopSpin 3.5 software. Spectra were visualized using the Numby and Matplotlib Python libraries.

**Magnetic resonance imaging**. Stock solutions of individually synthesized HoDy-TP1, DyDy-TP1, HoHo-TP1, and DyHo-TP1 compounds in 0.5 M MOPS/NaOH buffer (pH = 7.0) were pipetted into a 7 × 5-well plate (cut out of standard 384-well plate) and the volume in each well was completed with water to 75 μL to reach compound concentrations ∼0.36 mM each. The well plate was covered with plastic tape and imaged on a 4.7 T scanner Bruker Biospec 47/20 (Bruker BioSpin, Ettlingen, Germany) with a custom-built dual ¹H/¹⁹F RF surface coil. First, ¹H MRI was performed in all three planes (axial, coronal, and sagittal) for localization of the sample. Then, ¹⁹F NMR spectra were acquired by 90° single pulse sequence to precisely determine resonance frequencies of the compounds. ¹⁹F MRI images were obtained using a CSI sequence (120,000 scans, repetition time = 700 ms, field of view = 50 × 50 mm; slice thickness = 10 mm, matrix of acquired image = 16 × 16 × 512, matrix of reconstructed image = 64 × 64 × 512, acquisition time = 23 h 20 min, resolution frequency = 188,630,130 Hz, bandwidth = 9843 Hz/52.18 ppm). Images for display were prepared in Matlab software. First, the data in the Bruker format were imported to Matlab using read2dseq function by Cecil Yen (2021), https://www.mathworks.com/matlabcentral/fileexchange/69177-read_2dseq-quickly-reads-brucker’s-2dseq-mri-images). Maximum intensity projection along the frequency axis was used to find the peaks of the respective samples. Background signal (outside of main peaks) was calculated as an average of 41 slices (9 × 10–50 perpendicular to the frequency axis) and subtracted from each slice of the original data. Then, the intensity was normalized to 1 for the highest intensity. The final images displayed in Fig. 9 were obtained by averaging 15 slices (perpendicular to the frequency axis) around the maximum for each peak, and mapping the image intensity to specific color maps. Supplementary Movie 1 was created analogously, with each frame showing a floating average of 15 slices.

**Data fitting**. Fitting of experimental shifts δᵣ from statistical mixtures of M₁M₂-TP1 compounds to obtain δ₁, δ₂, δ₃ contributions was done according to Eq. (1) with the method of least squares using the Solver function in Microsoft Excel. Sequences of Dy³⁺/Ho³⁺ synthesized as individual compounds were the starting point for the assignment of δᵣ to particular M₁M₂ sequences. Next, homonuclear sequences were identified based on their repeated occurrences between mixtures containing the particular element. The assignment of heteronuclear sequences then contributed the other lanthanide ions. Therefore, δ₁, δ₂, δ₃ parameters for Sm³⁺ were fitted separately from the Sm³⁺/Y³⁺ statistical mixture of M₁M₂-TP1 compounds and were then used as fixed parameters in the global fit.

**Data availability**. The crystallographic data for the structures reported in this study have been deposited at the Cambridge Crystallographic Data Centre (CCDC) under deposition number 2072548 for [Dy(L₁)₃]·3H₂O and 2143481 for [Dy(L₂)₃]·3H₂O and can be obtained free of charge.
from the Centre via its website (www.ccdc.cam.ac.uk/getstructures). All other data supporting the findings in this study are available within the article and its Supplementary Information, as well as from the corresponding author upon request.

Code availability
No custom computer code was used in this study.

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Author contributions
M.P. conceived the project and supervised the work. J.K. synthesized the compounds. R.J. and M.K. designed and conducted the synthesis of the epoxide precursor. M.D. and O.S. conducted NMR measurements. D.J. and M.V. conducted MRI experiments and constructed a radiofrequency coil. T.D. created the artwork. M.P., J.K., and T.D. wrote the manuscript. All authors proofread and approved the manuscript.

Competing interests
M.P. and J.K. are co-inventors on a pending patent application no. PCT/CZ2020/050032 filed in the name of applicant Ustav Organické Chemie a Biochimie AV CR V.V.I. The application covers some of the compounds discussed in this work. The remaining authors declare no competing interests.

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